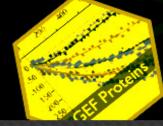
The Protein Experts

Cytoskeleton, Inc.

CYTOSKELETON, INC. MINICATALOG 2024

#### SIMPLIFYING THE COMPLEX

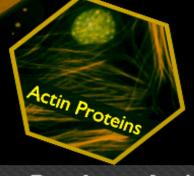
ACTIN PROTEINS TUBULIN REAGENTS SMALL G-PROTEIN TOOLS SIGNAL-SEEKER™ PTM KITS G-LISA™ ACTIVATION ASSAYS PULL-DOWN ACTIVATION ASSAYS GO-BLOT™ V2 WB PROCESSOR SPIROCHROME™ BIOPROBES MEMGLOW™ PM PROBES CUSTOM SERVICES MOTOR PROTEINS



Supporting the Scientific Community

for Over 30 Years

100% powered by wind and solar. 75% waste recycled



New Products Inside! Ubiquitination Detection Tools Purified Motor Proteins

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#### New Products - 3

Exciting new products to help you simplify the complex: discover the GO-Blot V2, Ubigitin kits, Motor Proteins, and LC probes

### Molecular Biology Tools - 5

ATPase, GTPase, and Phosphotase Biochem Kits.

#### Kinesins, Dynein, Myosin - 7

Pure and active kinesin and myosin family proteins, pre-formed microtubules, and F-actin used for motor substrates.

#### Activation Assays - 10, 11

Small GTPase Activation Assays offered in traditional pull-down bead format or advanced ELISA-based G-LISA® format.

#### Antibodies - 14

ytoskeleton.com

Highly characterized with validated applications. Developed in-house and tested for specificity and sensitivity.

## Actin Biochem Kits<sup>™</sup> - 16

Measure the effects of proteins and modulators on actin polymerization, and binding assays for F-actin.

#### Tubulin Biochem Kits<sup>™</sup> - 18

Measure the effects of proteins and compounds on tubulin and microtubule binding and polymerization.

At Cytoskeleton, we recognize the importance of our customers not only as scientists, but as a community of people working together towards a common goal: discovering new aspects of biology and providing a framework for understanding disease at the molecular and cellular levels. Conferences, events, and meetings are vital to creating this collaborative network of knowledge. Cytoskeleton is proud to sponsor and attend events across the globe in support of our community. We hope to see you out there in 2024!

#### Signal-Seeker ToolKits<sup>™</sup> - 4, 5

New Acetyl-Lysine, Ubiquitin, SUMO, and Phosphotyrosine Enrichment Kits for discovering new mechanisms of regulation.

#### Custom Services - 6

Compound screening, protein purification, and assay development services at an economical price.

#### Live Cell Imaging Tools - 8,9

Actin, ECM, DNA, lysosome and tubulin bioprobes, and small G-protein activators and inhibitors. Spirchrome<sup>™</sup> and Memglow<sup>™</sup>

#### Small G-protein Tools - 12, 13

New GEF proteins, inhibitors, activators (G-Switch modulators), antibodies, and affinity beads for active GTPase pull-down.

#### Actin Visualization - 15

Exceptionally bright and stable fluorescent phalloidins and Spirochrome<sup>™</sup> Bioprobes.

## Actin & ECM Proteins - 17

Pure and biologically active proteins, actin binding proteins, fluorescent and biotinylated actins, and antibodies.

#### Tubulin & FtsZ Proteins - 19

Biologically active proteins, fluorescent and biotin tubulin, antibodies, FtsZ proteins, and pre-formed microtubules.























NEV







# **New Products**

## GO-Blot V2





The GO-Blot<sup>™</sup> V2 Western Blot Processor is a reliable fluid delivery device for reproducibly probing blots and membranes with primary and secondary antibodies.

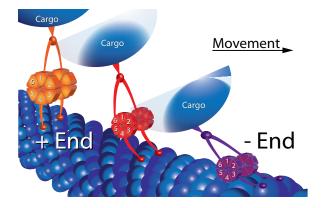
## Ubiquitination Detection Kits



- Comprehensive kits affinity & control beads, de-PTM inhibitors, validation antibody, lysis & wash buffers, etc.
- Measure endogenous signaling events.
- Analyze with western blots.

See p. 4-5 for more information

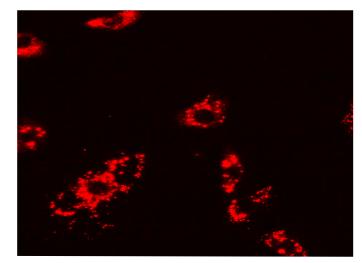
## Kinesin motors for screening and drug discovery



CENP-E Motor Domain Protein - (Cat. # CP01) Chromokinesin Motor Domain Protein - (Cat. # CR01) Eg5 Motor Domain Protein - (Cat. # EG01) MCAK Motor Domain Protein - (Cat. # MK01) MKLP1 Motor Domain Protein - (Cat. # MP01) MKLP2 Motor Domain Protein - (Cat. CS-MP05) KIF22 Motor Domain Protein - (Cat. # CS-KF02) KIF18A Motor Domain Protein - (Cat. # CS-KF18)

See p. 7 for more information

## LipiBright Lipid Droplet Probes



- Bright, fluorogenic live cell dyes that stain lipid droplets
- LipiBright probes are photostable and possess narrow absorption and emission bands; thus, multicolor imaging is possible.
- These probes exhibit ideal characteristics for microscopy and have been validated with multiple techniques including epifluorescent (widefield), confocal, and two photon.

#### See p. 9 for more information

cytoskeleton.com



# Signal-Seeker<sup>™</sup> ToolKits

## Novel Post-translational Modification Tools

- First to develop comprehensive PTM detection kits to simplify investigation for non-PTM experts.
- First to develop a universal lysis system which allows for the investigation of PTM crosstalk.
- First to develop a simple, genomic DNA removal filter that removes rather than shears DNA.
- First to develop a commercially availabe UBD that effectively enriches mono- and poly-ubiquitinated proteins.
- · First to develop an acetylation antibody that visualizes acetylated mitochondria by immunofluorescence.

#### Uses in Molecular Biology

- Use different kits to build a temporal protein regulation profile.
- Investigate the role of known protein modifications in your system.
- Detect endogenous levels (vs. transfected amounts) of modified proteins.

Protein regulation during signal transduction and other cellular events is, by necessity, a rapid and dynamic process. Most often, these mechanisms involve modification of an extremely small, but important fraction of the target protein. This makes the scientist's job of capturing key PTM regulatory processes difficult and frustrating.

At Cytoskeleton, we have focused on generating accurate methods to measure these small, endogneous changes; because it is critical to determine if these PTM events are occuring physiologically.

## Be The First To Discover Mono- And Poly-Ubiquitination Of Your Protein

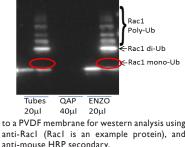
The only commercially available UBD that effectively enriches mono- and poly-ubiquitinated proteins

No heavy and light chain interfence, which occurs with antibody enrichment

#### Learn more at: https://www.cytoskeleton.com/uba01-beads Cytoskeleton Alternative UBDs ENZO Lifesenso IP Beads IP Bead IP Bead Rac1 oly-Ub olv-Ub c1 di-Ub mono-Uh Tubes QAP ENZC UBA01 20µl

Legend: Ubiquitin affinity beads (Cat. # UBA01beads) were used to precipitate ubquitinated proteins from A431 cell extracts. Products were run on 4-20% SDS-PAGE and transfered

<u>cytoskeleton.com</u>



anti-Racl (Racl is an example protein), and anti-mouse HRP secondary.

## A Treasure Trove of PTM Resources and Information

Cytoskeleton has prepared a wealth of material to assist with your PTM research. From regular newsletters, tech tips, and videos Cytoskeleton.com is a great place to learn about PTMs and how our Signal-Seeker Tools can assist in your PTM discovery. Visit https://www. cytoskeleton.com/signal-seeker-information-resources to start learning.







#### SUMOylation 1 Products

| Description                        | Amount     | Cat. #      |
|------------------------------------|------------|-------------|
| Signal-Seeker™ SUMO1 Detection Kit | 30 assays  | BK165       |
| Signal-Seeker™ SUMO1 Detection Kit | 10 assays  | BK165-S     |
| SUMO1 Affinity Beads               | 30 assays  | ASM11-beads |
| SUMO1 Control Beads                | 10 assays  | CIG03-beads |
| SUMO1 Mouse Antibody (5D8B16)      | 1 x 100 μl | ASM01       |

#### SUMOylation 2/3 Products

| Description                           | Amount     | Cat. #      |
|---------------------------------------|------------|-------------|
| Signal-Seeker™ SUMO 2/3 Detection Kit | 30 assays  | BK162       |
| Signal-Seeker™ SUMO 2/3 Detection Kit | 10 assays  | BK162-S     |
| SUMO 2/3 Affinity Beads               | 20 assays  | ASM24-beads |
| Mouse IgG Control                     | 10 assays  | CIG01-beads |
| SUMO 2/3 Mouse Antibody (12F3)        | 2 x 100 μl | ASM23       |
| SUMO 2/3 Mouse Antibody (11G2)        | 2 x 200 μl | ASM24       |
| SUMO 2/3 Mouse Antibody-HRP labeled   | 1 x 100 μl | ASM23-HRP   |

#### **Phosphotyrosine Products**

| Description                                  | Amount     | Cat. #      |
|--|------------|-------------|
| Signal-Seeker™ Phosphotyrosine Detection Kit | 30 assays  | BK160       |
| Signal-Seeker™ Phosphotyrosine Detection Kit | 10 assays  | BK160-S     |
| Phosphotyrosine Affinity Beads               | 40 assays  | APY03-beads |
| Mouse IgG Control                            | 10 assays  | CIG01-beads |
| Phosphotyrosine Mouse Antibody (11G2)        | 2 x 100 μl | APY03       |
| Phosphotyrosine Antibody-HRP labeled         | 1 x 100 μl | APY03-HRP   |

#### **Acetyl-Lysine Products**

| Description                                | Amount     | Cat. #      |
|--|------------|-------------|
| Signal-Seeker™ Acetyl-Lysine Detection Kit | 30 assays  | BK163       |
| Signal-Seeker™ Acetyl-Lysine Detection Kit | 10 assays  | BK163-S     |
| Acetyl-Lysine Affinity Beads               | 40 assays  | AAC04-beads |
| Acetyl-Lysine Control Beads                | 10 assays  | CIG02-beads |
| Acetyl-Lysine Mouse Antibody (3C6.08.20)   | 1 x 200 μl | AAC01       |
| Acetyl-Lysine Mouse Antibody (7B5A1)       | 2 x 100 μl | AAC02       |
| Acetyl-Lysine Mouse Antibody (19C4B2.1)    | 2 x 100 μl | AAC03       |
| Acetyl-Lysine Mouse Antibody-HRP labeled   | 1 x 100 μl | AAC03-HRP   |

#### **Ubiquitin Products**

| Description                                 | Amount     | Cat. #      |
|---|------------|-------------|
| Signal-Seeker™ Ubiquitination Detection Kit | 30 assays  | BK161       |
| Signal-Seeker™ Ubiquitination Detection Kit | 10 assays  | BK161-S     |
| Ubiquitination Affinity Beads               | 40 assays  | UBA01-beads |
| Ubiquitination Control beads                | 10 assays  | CUB02-beads |
| Ubiquitin Mouse Antibody                    | 2 x 100 μl | AUB01       |
| Ubiquitin Mouse Antibody-HRP labeled        | 1 x 100 μl | AUB01-HRP   |

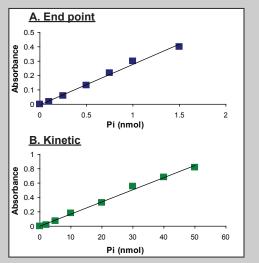
#### BlastR<sup>™</sup> Lysis System

| Description                        | Amount    | Cat. # |
|------------------------------------|-----------|--------|
| BlastR™ Rapid Lysate Filter System | 50 assays | BLR01  |
| BlastR™ Rapid Lysate Filters       | 50 assays | BLR02  |
| BlastR™ Lysis/Dilution Buffer Kit  | 50 lyses  | BLR03  |

## ATPase, GTPase, & Phosphatase Biochem Kits<sup>\*\*</sup>

ATPases, GTPases, and other phosphatases liberate inorganic phosphate (Pi) from their respective triphosphate nucleotide or substrate. BK051-BK054 are suitable for HTS applications. BK051-BK054 and BK060 measure free phosphate via binding to a reporter dye or by enzymatic conversion into a reporter molecule. BK053 and BK054 are end-point assays suitable for measuring microtubule-induced kinesin ATPase or F-actin-induced myosin ATPase activity. BK051, BK052 and BK060 are kinetic assays, thus suitable for Vmax or Kcat determinations. These kits require a higher level activity ATPase or GTPase for sufficient sensitivity. BK060 is specialized for kinesins.

| Phosphate Quantitation Biochem Kits™   | Cat. #    | Amount      |
|--|-----------|-------------|
| ATPase ELIPA™ (enzyme-linked, colorimetric)<br>Kinetic quantitation of ATP hydrolysis (Kcat 0.05 to >1.0)  | BK051/052 | 96 assays   |
| CytoPhos™ Phosphate Assay (endpoint assay)<br>Colorimetric assay for ATPases & GTPases (Kcat 0.01 to >1.0) | BK054     | 1000 assays |
| GTPase ELIPA™ (enzyme-linked, colorimetric)<br>Kinetic quantitation of GTP hydrolysis (Kcat 0.05 to >1.0)  | BK051/052 | 96 assays   |
| Kinesin ELIPA <sup>™</sup> Biochem Kit<br>For real time kinetic and Vmax kinesin ATPase measurements       | BK060     | 96 assays   |
| Kinesin ATPase Endpoint Assay<br>For endpoint measurement of kinesin ATPase activity                       | BK053     | 1000 assays |
| Purine Nucleoside Phosphorylase protein<br>Catalyzes the transfer of phosphate to MSEG reporter            | ELP03     | 96 assays   |

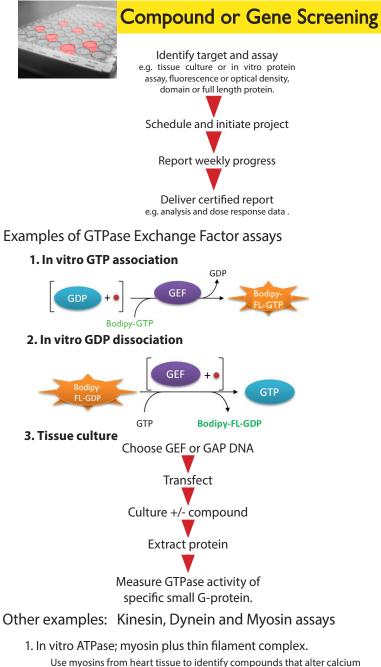


Comparison of standard curves of Cytoskeleton's endpoint (BK053 and BK054) and kinetic (BK051/52 and BK060) phosphate assays. Endpoint assays have a linear response between 0.1 and 1.5 nmol Pi. Kinetic assays give a linear response between 2 and 50 nmol Pi.

Custom Services

#### About Custom Services

Like our product offerings, the Custom Services department emphasizes quality products and services. We also understand *accuracy* and *timeliness* are critical elements for a successful project. The process starts with an experienced scientist asking for *specifications and success factors* for your project. Within 24 hours, the quotation will arrive and work can

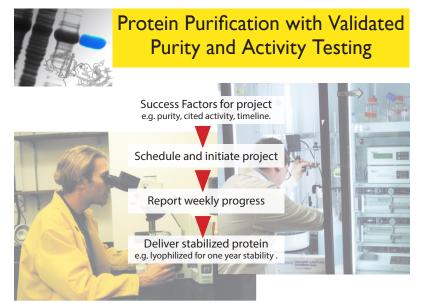


- Use myosins from heart tissue to identify compounds that alter calciu induced ATPase of myosin on thin filaments. 2. In vitro ATPase: kinesin plus microtubules.
- Use purified kinesins that are important in tumor cells to identify compounds that inhibit cancer cell movement or division.
- 3. In vitro ATPase; dynein plus microtubules.

Use dynein isolated from neuronal tissue to identify compounds that increase dynein activity as a way to improve the outcome of dementia diseases.



start at the next available schedule date. Regular updates are provided until project completion. Once complete, we continue support through timely citation-based advice and practical experience. Choose from over forty defined modules (full list is available online).



#### Many satisfied customers, including:

Novartis, Merck, Biokinesis, Amgen, Bayer Cropsciences, Alcon, Frost Biologic, Cullinan Pharmaceuticals, Sigma-Clermont Institute, Imperial Cancer Research Institute, and many other diagnostic companies requiring active protein targets!

## Example purified proteins:

| Ras Small-G Proteins                                 | Motor Proteins                                 |
|--|--|
| K-Ras4B Protein, hu. rec., wild-type (Cat. # RS03)   | MKLP2 kinesin like protein (Cat. # CS-KF51)    |
| K-Ras4B Protein, hu. rec., G12V mutant (Cat. # RS04) | Dynein neuronal tissue (Cat. CS-DN01)          |
| K-Ras4B Protein, hu. rec., other mutants (inquire)   | S1 myosin, cardiac tissue (Cat. # CS-MYS03)    |
| R-Ras Protein, hu. rec., wild type (Cat. # RS05)     | S1 myosin, smooth muscle (Cat. # CS-MYS05      |
| H-Ras Protein, hu. rec., wild-type (Cat. # RS01)     | S1 myosin, skeletal muscle (Cat. # CS-MYS04    |
| GEF Proteins   | Thin filament complex cardiac (Cat. # TFC01)   |
| SOS1 Exchange Factor (Cat. # CS-GE02)                | Thin filament complex skeletal (Cat. # TFC02)  |
| Tiam1 (Rac GTP) Exchange Factor (Cat. # CS-GE04)     | Heavy meromyosin cardiac tissue (Cat. # MH03)  |
| av2 (Rac GTP) Exchange Factor (Cat. # CS-GE06)       | NEW Fascin-1 Protein: Wild-Type(Cat. CS-FSC01) |

NEW KIF18A Motor Domain (I-374) His-Protein: Wild-Type (Human Recombinant) (Cat # CS-KF18)

Absorbance (360

Product Uses:

- Measurement of microtubuleactivated ATPase assays
- Identification/characterization of proteins or small molecules that affect motor ATPase activity.
- Identification/characterization of proteins or small molecules that affect motor/microtubule interactions.

Legend: KIFI8A microtubule ATPase activity determined with Kinesin ELIPA Kit (Cat#. BK060)

For more infor<u>mation about Protein Purification Services please</u> <u>visit www.cytoskeleton.com/custom-services</u> or email tservice@cytoskeleton.com



Figure 1: Schematic diagram of dynein pulling cargo along a microtubule.

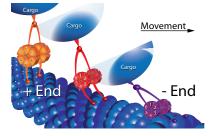
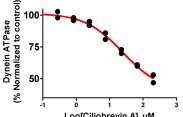


Figure 2: Dose response curve of Ciliobrevin A inhibiting cytoplasmic dynein.



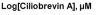


Figure 1: Schematic representation of proposed dynein torsion gear mechanism and microtubule (MT) catch-bonding. The six member ring of the cytoplasmic dynein complex's (CDC) motor domain is shown in a relaxed state in orange and during high load conditions the ring is compressed as shown by the red model. During very high load conditions, CDC's microtubule binding

domain clamps down onto the MT as shown by the purple model.

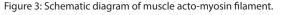
Figure 2: Dynein (Cat. # DN01) is available from Cytoskeleton as a purified complex that has microtubule stimulated ATPase activity which is inhibited by Ciliobrevin A with an ICso of 30  $\mu$ M, which is similar to Firestone et al. (2012, Fig. 4).

#### Myosin & Thin Filament Proteins

| •   |         |        |                    |                       |
|---|---------|--------|--------------------|-----------------------|
| Myosin Proteins   | Source  | Purity | Cat. #             | Amount                |
| Myosin S1 fragment (cardiac)  | Bovine  | >85%   | CS-MYS03           | 1 x 250 µg            |
| Myosin S1 fragment (skeletal)   | Rabbit  | >85%   | CS-MYS04           | 1 x 250 µg            |
| Myosin S1 fragment (smooth)   | Chicken | >85%   | CS-MYS05           | 1 x 250 µg            |
| Myosin II Skeletal Muscle Protein   | Rabbit  | >95%   | MY02-A<br>MY02-B   | 5 x 1 mg<br>20 x 1 mg |
| Myosin Cardiac Muscle Protein   | Bovine  | >95%   | MY03-A<br>MY03-B   | 5 x 1 mg<br>20 x 1 mg |
| Heavy Meromyosin Skeletal Muscle<br>Protein   | Rabbit  | 80%    | MH01-A             | 4 x 50 μg             |
| Heavy Meromyosin Cardiac Muscle<br>Protein  | Bovine  | 80%    | CS-MH03            | 1 x 100 µg            |
| Pre-formed F-actin filaments  | Rabbit  | >99%   | AKF99-A<br>AKF99-B | 1 x 1 mg<br>5 x 1 mg  |
| Actin Thin Filaments (cardiac)<br>Calcium sensitive complex of F-actin,<br>tropomyosin $\alpha/\beta$ & Troponin C,I,T  | Bovine  | >90%   | TFC01              | 1 x 1 mg              |
| Actin Thin Filaments (skeletal)<br>Calcium sensitive complex of F-actin,<br>tropomyosin $\alpha/\beta$ & Troponin C,I,T | Rabbit  | >90%   | CS-TFC02           | 1 x 1 mg              |
| <b>Tropomyosin / Troponin Complex</b><br>Cardiac tropomyosin $\alpha/\beta$ & Troponin C,I,T                            | Bovine  | >60%   | CS-TT05            | 1 x 1mg               |

#### Pre-formed Microtubules & F-Actin Reagents

| Microtubules and Other Reagents   | Cat. #              | Amount                  |
|---|---------------------|-------------------------|
| Thin Filament Protein (cardiac tropomyosin/tropomodulin/<br>actin, Ca <sup>2+</sup> activated myosin ATPase       | TFC01               | 1 x 1 mg                |
| Thin Filament Protein (skeletal mus. tropomyosin/<br>tropomodulin/actin, Ca <sup>2+</sup> activated myosin ATPase | CS-TFC02            | 1 x 1 mg                |
| Microtubules, Pre-formed, lyophilized, porcine source, substrate for kinesin ATPase assays                        | MT002-A<br>MT002-XL | 4 x 500 μg<br>1 x 10 mg |
| Actin Filaments, Pre-formed, lyophilized<br>A ready to use substrate for myosin ATPase assays                     | AKF99-A<br>AKF99-B  | 1 x 1 mg<br>5 x 1 mg    |
| Paclitaxel (2 mM) Stabilizes microtubules   | TXD01               | 10 x 100 µl             |



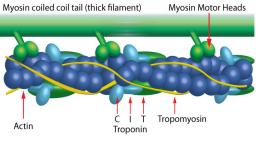


Figure 4: Calcium induced ATPase activity from a reconstituted acto-myosin filament.

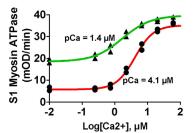


Figure 4: Actin thin filament protein (Cat. # TFC01) was mixed with S1-myosin (Cat. # MYS03) to re-create the acto-myosin filament *in vitro*. Calcium was titrated between 0.2 and 60  $\mu$ M and ATPase rate was measured kinetically at OD<sub>360nm</sub> using the components of the ATPase ELIPA Kit (Cat. # BK051, pg. 18). The concentration of calcium was plotted against the rate of ATPase activity to produce the dose

response curves. Red line denotes the control with a pCa =  $4.1 \, \mu$ M which is similar to published pCa values for reconstituted cardiac sarcomeres decribed in Holroyde et al. (1980, Fig. 6). This system is responsive to compounds that bind to the myosin motor domain, e.g. Omecavit mercarbil with a modified pCa =  $1.4 \, \mu$ M (green line), and hence it can be used as a screening tool to develop new cardiac therapeutic drugs.

#### Kinesin & Dynein Proteins

NEW

NEW

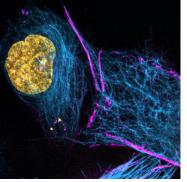
NEW

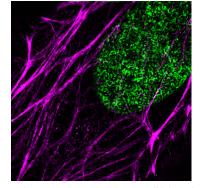
| Kinesin & Dynein Proteins                             | Source           | Purity | Cat. #                      | Amount                              |
|---|------------------|--------|-----------------------------|-------------------------------------|
| CENP-E Motor Domain Protein                           | H. sapiens       | >85%   | CP01-A<br>CP01-XL           | 2 x 25 μg<br>1 x 1 mg               |
| Chromokinesin Motor Domain<br>Protein                 | H. sapiens       | >85%   | CR01-A                      | 2 x 25 μg                           |
| Dynein (cytoplasmic)                                  | Porcine<br>brain | >80%   | CS-DN01                     | 1 x 50 µg                           |
| Eg5 Motor Domain Protein                              | H. sapiens       | >85%   | EG01-A<br>EG01-B<br>EG01-XL | 2 x 25 μg<br>10 x 25 μg<br>1 x 1 mg |
| Eg5 Homolog BimC Motor<br>Domain Protein              | A. nidulans      | >85%   | BM01-A                      | 2 x 25 µg                           |
| Eg5 Homolog BimC Motor<br>Domain Protein              | A. fumigatus     | >85%   | EG02-A                      | 2 x 15 µg                           |
| KIFC3 Motor Domain Protein                            | H. sapiens       | >85%   | KC01-A                      | 2 x 25 µg                           |
| KIF3C Motor Domain Protein                            | H. sapiens       | >85%   | KF01-A                      | 2 x 25 µg                           |
| KIF7 motor domain                                     | H. sapiens       | >85%   | CS-KF51                     | 1 x 100 µg                          |
| Kinesin Heavy Chain Motor<br>Domain Protein           | H. sapiens       | >85%   | KR01-A<br>KR01-XL           | 2 x 25 μg<br>1 x 1 mg               |
| MCAK Motor Domain Protein                             | H. sapiens       | >85%   | MK01-A                      | 2 x 25 µg                           |
| MKLP1 Motor Domain Protein                            | H. sapiens       | >85%   | MP01-A<br>MP01-XL           | 2 x 25 μg<br>1 x 1 mg               |
| MKLP2 Motor Domain Protein                            | H. sapiens       | >85%   | CS-MP05                     | 1 x 50 µg                           |
| KIF22 Motor Domain<br>(5-378) His-Protein: wild-type  | H. sapiens       | 90%    | CS-KF02                     | 1 x 100 µg                          |
| KIF18A Motor Domain<br>(1-374) His-Protein: wild-type | H. sapiens       | 80%    | CS-KF18                     | 1 x 100 µg                          |
| KIF7 Motor Domain Protein                             | H. sapiens       | >80%   | CS-KF51                     | 1 x 100 µg                          |

# Live Cell Imaging Reagents

#### Spirochrome's New SPY probes - Advantages:

- Increased cell membrane permeability
- Verapamil no longer needed for consistent staining
- Improved compatibility across more cell lines
- Less cytotoxic than SiR probes
- Increased spectra range: FITC, TRITC, and Texas Red





Left : 93X STED-imaged HeLa cell labeled with SPY555-actin (magenta), SPY595-DNA (orange),and SPY650-tubulin (blue). Image provided courtesy of Spirochrome.

Right : 93X STED-imaged HeLa cell labeled with SPY505-DNA (green) and SPY555-actin (magenta). Image provided courtesy of Spirochrome.

## Small G-protein Modulators and Actin Imaging

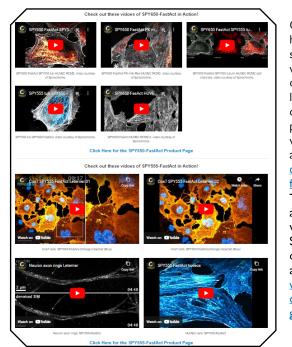
|              | Cat. #   | Amount  |
|--------------|--|---|
| 690 / 720 nm | CY-SC013   | 35 nmol   |
| 630 / 680 nm | CY-SC001   | 50 nmol   |
| 555 / 580 nm | CY-SC202   | 100 Stains  |
| 619 / 636 nm | CY-SC402   | 100 Stains  |
| 535 / 590 nm | APHR-A<br>APHR-C   | 4 x 10 μg<br>20 x 10 μg   |
| 535 / 590 nm | AR05-B<br>AR05-C   | 10 x 20 μg<br>20 x 20 μg  |
|              | CN03-A<br>CN03-B   | 3 x 20 μg<br>9 x 20 μg  |
|              | CT04-A<br>CT04-B<br>CT04-C   | 1 x 20 μg<br>5 x 20 μg<br>20 x 20 μg  |
|              | CN04-A<br>CN04-B   | 3 x 20 μg<br>9 x 20 μg  |
|              | CN01-A<br>CN01-B   | 5 x 10 units<br>20 x 10 units   |
|              | CN02-A<br>CN02-B   | 5 x 10 units<br>20 x 10 units   |
|              | 690 / 720 nm<br>630 / 680 nm<br>555 / 580 nm<br>619 / 636 nm<br>535 / 590 nm | 690/720 nm       CY-SC013         630/680 nm       CY-SC001         555/580 nm       CY-SC402         619/636 nm       CY-SC402         535/590 nm       APHR-A<br>APHR-C         535/590 nm       AR05-B<br>AR05-B         535/590 nm       AR05-B<br>AR05-C         610       CN03-A<br>CN03-B         610       CN04-B<br>CN04-B         610       CN04-A<br>CN01-B         610       CN02-A |

## ECM Imaging

| Description                                       | Ex / Em      | Cat. #  | Amount     |
|---|--------------|---------|------------|
| Fibronectin                                       | 535 / 590 nm | FNR01-A | 5 x 20 μg  |
| Red fluorescent, rhodamine                        |              | FNR01-B | 20 x 20 μg |
| Fibronectin                                       | 460 / 520 nm | FNR02-A | 5 x 20 μg  |
| Green fluorescent, HiLyte Fluor <sup>**</sup> 488 |              | FNR02-B | 20 x 20 μg |
| <b>Laminin</b>                                    | 535 / 590 nm | LMN01-A | 5 x 20 μg  |
| Red fluorescent, rhodamine                        |              | LMN01-B | 20 x 20 μg |
| <b>Laminin</b>                                    | 460 / 520 nm | LMN02-A | 5 x 20 μg  |
| Green fluorescent, HiLyte Fluor <sup>™</sup> 488  |              | LMN02-B | 20 x 20 μg |

#### Spirochrome's Newest Generation Probe Is Here! SPY650-FastAct™ Dynamic Actin Labeling

SPY650-FastAct<sup>™</sup> is a bright, far red, fluorogenic & non toxic F-actin stain based on our SPY<sup>™</sup> dyes series. Its optimized structure allows to label F-actin in live cells with high specificity and low background. The unique and unmatched feature of SPY650-FastAct<sup>™</sup> is its ability to label very fast actin dynamics. The probe does not require any genetic manipulation, transfection or overexpression of fluorescent proteins.



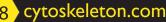
Cytoskeleton has prepared a series of videos which display the dynamic actin labeling potential of the FastAct™ probes. You can view these videos at https://www. cytoskeleton.com/ fastactvideogallery. There is also a collection of videos showing Spirochrome's other probes in action at <a href="https://">https://</a> www.cytoskeleton. com/spirochromegallery.

## NEW SPY-FastAct™ Dynamic Actin Labeleing

| Description  | Ex / Em | Cat. #   | Amount  |
|--|---------|----------|---------|
| <b>SPY555-FastAct™</b><br>Dynamic Actin Labeling Probe | 555/580 | CY-SC205 | 50 nmol |
| <b>SPY650-FastAct™</b><br>Dynamic Actin Labeling Probe | 652/674 | CY-SC505 | 50 nmol |
| SPY700-FastAct™<br>Dynamic Actin Labeling Probe        | 696/718 | CY-SC605 | 50 nmol |

## DNA Imaging

| 00  |              |          |            |
|---|--------------|----------|------------|
| Description   | Ex / Em      | Cat. #   | Amount     |
| SiR-DNA Kit<br>Includes SiR-DNA and Verapamil       | 630 / 680 nm | CY-SC007 | 50 nmol    |
| SiR700-DNA Kit<br>Includes SiR700-DNA and Verapamil | 690 / 720 nm | CY-SC015 | 35 nmol    |
| SPY505-DNA<br>Includes SPY505-DNA Probe             | 512 / 531 nm | CY-SC101 | 100 stains |
| SPY555-DNA<br>Includes SPY555-DNA Probe             | 555 / 580 nm | CY-SC201 | 100 stains |
| SPY595-DNA<br>Includes SPY595-DNA Probe             | 599 / 615 nm | CY-SC301 | 100 stains |
| SPY620-DNA<br>Includes SPY620-DNA Probe             | 619 / 636 nm | CY-SC401 | 100 stains |
| SPY650-DNA<br>Includes SPY650-DNA Probe             | 652 / 674 nm | CY-SC501 | 100 stains |
| SPY700-DNA<br>Includes SPY700-DNA Probe             | 696 / 718 nm | CY-SC601 | 100 stains |
|   |              |          |            |

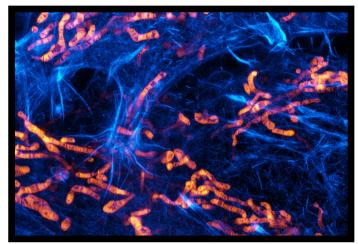


Live Cell Imaging Reagents



## Spirochrome: PKmito<sup>™</sup> Probes

We are proud to announce that the PKmito<sup>™</sup> probes for gentle imaging of mitochondria in live cells have been developed and published by the lab of Zhixing Chen from Peking University. They were carefully designed to reduce the generation of reactive oxygen species during imaging and allow long-term imaging or timelapse STED imaging in live cells.



Cell Stained with PKmito Orange

#### NEW PKmito - Mitochondria Imaging

| Product         | Ex/Em      | Cat. #   | Amount     |
|-----------------|------------|----------|------------|
| PKmito DEEP RED | 644/670nm  | CY-SC055 | 100 stains |
| PKmito ORANGE   | 591/608 nm | CY-SC053 | 100 stains |
| PKmito RED      | 549/569 nm | CY-SC052 | 100 stains |

#### Membrane Sensor - Imaging Probes

| Description   | Ex / Em | Cat. #   | Amount  |
|---|---------|----------|---------|
| <b>ER Flipper-TR Kit</b><br>For fluorescence cell membrane microscopy   | 480/600 | CY-SC021 | 50 nmol |
| Flipper-TR Kit<br>For fluorescence cell membrane microscopy             | 480/600 | CY-SC020 | 50 nmol |
| <b>Mito Flipper-TR Kit</b><br>For fluorescence cell membrane microscopy | 480/600 | CY-SC023 | 50 nmol |

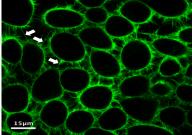
#### **Tubulin Imaging**

| Description   | Ex / Em      | Cat. #               | Amount                  |
|---|--------------|----------------------|-------------------------|
| SiR700-Tubulin Kit<br>Includes SiR700-Tubulin and Verapamil | 690 / 720 nm | CY-SC014             | 35 nmol                 |
| SiR-Tubulin Kit<br>Includes SiR-Tubulin and Verapamil       | 630 / 680 nm | CY-SC002             | 50 nmol                 |
| SPY555-Tubulin<br>Includes SPY555-Tubulin Probe             | 555 / 580 nm | CY-SC203             | 100 stains              |
| SPY650-Tubulin<br>Includes SPY650-Tubulin Probe             | 652 / 674 nm | CY-SC503             | 100 stains              |
| AMCA Labeled Tubulin  | 350 / 440 nm | TL440M-A<br>TL440M-B | 5 x 20 μg<br>20 x 20 μg |
| HiLyte Fluor™ 488<br>Labeled Tubulin                        | 460 / 520 nm | TL488M-A<br>TL488M-B | 5 x 20 μg<br>20 x 20 μg |
| TRITC Rhodamine<br>Labeled Tubulin                          | 535 / 590 nm | TL590M-A<br>TL590M-B | 5 x 20 μg<br>20 x 20 μg |
| X-Rhodamine<br>Labeled Tubulin                              | 560 / 620 nm | TL620M-A<br>TL620M-B | 5 x 20 μg<br>20 x 20 μg |
| HiLyte Fluor™ 647<br>Labeled Tubulin                        | 620 / 670 nm | TL670M-A<br>TL670M-B | 5 x 20 μg<br>20 x 20 μg |

#### MemGlow<sup>™</sup> Plasma Membrane Probes for Bioimaging

#### Features and advantages of MemGlow<sup>™</sup> probes:

- Bright: efficient labeling of filopodia and nanotube at nanomolar concentrations
- Fluorogenic: Utilize cyanine or BODIPY dyes with zwitterionic membrane anchor groups
- Non-toxic: does not alter biological sample, while permitting longterm imaging
- Simple staining protocol: compatible with live cells, fixed cells, and tissue



The plasma membrane of live KB cells labeled with 20 nM MemGlow<sup>™</sup> 488 and imaged with laser scanning confocal microscopy. Intercellular filopodia and nanotubes are visible between cells throughout (white arrows). Image provided courtesy of Mayeul Collot et al, CNRS, Paris, France.

#### MemGlow<sup>™</sup> Probes

| Product                                 | Cat. #             | Amount            |
|---|--------------------|-------------------|
| MemGlow™ 488 Fluorogenic Membrane Probe | MG01-02<br>MG01-10 | 2 nmol<br>10 nmol |
| MemGlow™ 560 Fluorogenic Membrane Probe | MG02-02<br>MG02-10 | 2 nmol<br>10 nmol |
| MemGlow™ 590 Fluorogenic Membrane Probe | MG03-02<br>MG03-10 | 2 nmol<br>10 nmol |
| MemGlow™ 640 Fluorogenic Membrane Probe | MG04-02<br>MG04-10 | 2 nmol<br>10 nmol |
| MemGlow™ 700 Fluorogenic Membrane Probe | MG05-02<br>MG05-10 | 2 nmol<br>10 nmol |

#### MemGlow<sup>™</sup> Nile Red Polarity Probes

| Product                       | Cat. # | Amount |
|-------------------------------|--------|--------|
| NR4A Membrane Polarity Probe  | MG06   | 2 nmol |
| NR12A Membrane Polarity Probe | MG07   | 4 nmol |
| NR12S Membrane Polarity Probe | MG08   | 4 nmol |

#### LipiBright<sup>™</sup> Fluorogenic Lipid Droplet Probes

Cytoskeleton is proud to announce the release of three new, bright fluorogenic probes to visualize lipid droplets. LipiBright™ live cell probes fluoresce 300 to 1000 times brighter in an oily/lipid environment compared to aqueous solutions and effectively label lipid droplets.

#### Advantages:

I. LipiBright probes are photostable and possess narrow absorption and emission bands, a distinct advantage over the traditional Nile Red probe; thus, multicolor imaging is possible.

2. These probes exhibit ideal characteristics for microscopy and have been validated with multiple techniques including epifluorescent (widefield), confocal, and two photon.

#### ▶ LipiBright<sup>™</sup> Probes

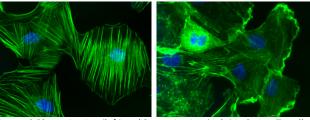
| Product                             | Cat # | Amount  |
|-------------------------------------|-------|---------|
| LipiBright <sup>™</sup> 530 SMCy3.5 | MG11  | 70 nmol |
| LipiBright <sup>™</sup> 650 SMCy5.5 | MG12  | 70 nmol |
| LipiBright <sup>™</sup> 740 SMCy7.0 | MG13  | 70 nmol |



#### About Activation Assays

Since 2001, Cytoskeleton has provided the scientific community with the most robust, accurate, and time-saving kits to measure Small GTP-binding protein (SmG) activation. Along the way, we have developed numerous versions for different SmGs, such as Rho, Rac, Arf1 & 6, Ras, Cdc42, and Ral. Also, the quantifiable G-LISA versions enabled a new wave of more sensitive applications, e.g. measurement in limited primary cell numbers and Matrigel 3D matrices. We continue to develop and maintain these high standards, which allow you to produce the best results in the least amount of time.

SmGs are involved in regulating cell signaling pathways and impact a wide range of cellular processes, functions, and morphology. The pull-down version of the assay uses affinity beads which are incubated with the extract and then separated by centrifugation. The pelleted products are separated by SDS-PAGE and blotted onto a membrane for Western analysis of the SmG of interest. The G-LISA® format is a modified ELISA which has the affinity reagent permanently attached to the well of a 96-well plate. The extract is incubated in the well which is then washed and probed with primary and secondary antibodies.



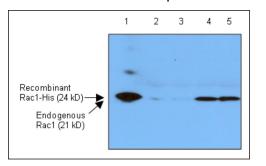
Legend: Rho activation (left) and Rac activation (right) in Swiss 3T3 cells. F-actin is visualized with fluorescent green phalloidin staining (Cat.# PHDG1) and nuclear blue DNA staining with DAPI. Cells were activated with Cat.# CN03 (left) and Cat. # CN04 (right).

#### Comparison of Pull-down and G-LISA formats

| Parameter                            | Pull-down        | G-LISA®    |
|--------------------------------------|------------------|------------|
| Total protein per assay              | 500-2000 μg      | 10-50 μg   |
| Assay time                           | 10-12 h (2 days) | <3 h       |
| Primary cells & 3D matrix compatible | No               | Yes        |
| Sample handling                      | 10 Samples       | 96 Samples |
| Quantitative data*                   | Semi             | Yes        |

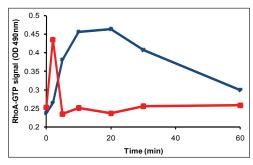
\* Numerical readouts and fewer sample handling steps make G-LISA® assays more quantitative.

#### Pull-down Result Example



Swiss 3T3 cells were serum-starved for 24h; after this, a sample was treated with 10 ng/ml of EGF for 2 min (Lanes 4 & 5). Other cells were not treated and remained serum-starved (Lanes 2 & 3). Rac1 activation was measured using the Rac1 Activation pull-down assay. 500  $\mu$ g of lysate were assayed with 10  $\mu$ g of PAK-PBD beads (Lanes 2-5). Lane 1 shows 20 ng of recombinant Rac1-His protein run as a Western blot standard.

#### G-LISA<sup>®</sup> Result Example



Time course of activation of RhoA in Swiss 3T3 cells by CN01 and LPA. Serum-starved Swiss 3T3 cells were treated with Rho Activator I, Cat. # CN01 (blue diamonds) or LPA (red squares). RhoA activity was measured by reading signals at OD<sub>490nm</sub>. Data are background subtracted.



#### Example Product Citations RhoA G-LISA® (Cat. # BK124)

Nath, Anu S. et al. Cell Reports 2022 ISSN 2211-1247 https://www.sciencedirect.com/science/article/pii/ S2211124722001607 Swaminathan, Bhairavi et al. Scientific Reports 2022 https://www.nature.com/articles/s41598-022-05666-1

#### Rac1 G-LISA® (Cat. # BK126)

Nath, Anu S. et al. Cell Reports 2022 https:// www.sciencedirect.com/science/article/pii/ S2211124722001607 Berthenet K. et al. Cell Rep. 2020 Jun 9;31(10):107731. doi: 10.1016/j.celrep.2020.107731.

#### Rac1 G-LISA® (Cat. # BK128)

Kim, Seunghoon et al. Frontiers in Immunology 2022 https://www.frontiersin.org/articles/10.3389/ fimmu.2022.850287/full Borza, Corina M. et al. Frontiers in Cell and Developmental Biology 2022 https://www.ncbi.nlm. nih.gov/pmc/articles/PMC8928223/

#### Cdc42 G-LISA® (Cat. # BK127)

Jiang, Ruiwei et al. Cells Death & Disease 2022 https:// www.nature.com/articles/s41419-022-05184-y Meng, Zhipeng et al. Science Advances 2022 https:// www.science.org/doi/10.1126/sciadv.abl9806







#### Pull-down Activation Assays

Pull-down assays utilize affinity beads linked to an effector protein that selectively binds active GTPase followed by quantitation with Western blotting.

| Pull-down Activation Assays                            | Cat. #           | Amount                 |
|--|------------------|------------------------|
| Combo RhoA/Rac1/Cdc42 Activation Assay<br>Biochem Kit™ | BK030            | 3 x 10 assays          |
| Arf1 Activation Assay Biochem Kit™                     | BK032-S          | 20 assays              |
| Arf6 Activation Assay Biochem Kit™                     | BK033-S          | 20 assays              |
| Cdc42 Activation Assay Biochem Kit™                    | BK034-S<br>BK034 | 20 assays<br>50 assays |
| Rac1 Activation Assay Biochem Kit™                     | BK035-S<br>BK035 | 20 assays<br>50 assays |
| RalA Activation Assay Biochem Kit™                     | BK040            | 50 assays              |
| Ras Activation Assay Biochem Kit™                      | BK008-S<br>BK008 | 20 assays<br>50 assays |
| RhoA Activation Assay Biochem Kit™                     | BK036-S<br>BK036 | 20 assays<br>80 assays |



| Cytoskeleton has compiled          |
|------------------------------------|
| useful information about           |
| Activation Assays and              |
| Small G-Proteins which             |
| can be viewed at : <u>https://</u> |
| www.cytoskeleton.com/              |
| small-g-protein-information-       |
| resources-d1                       |



## G-LISA<sup>®</sup> Activation Assays

G-LISAs use a 96-well plate coated with effector protein that selectively binds the active GTPase followed by quantitation with ELISA techniques.

| G-LISA Activation Assays   | Cat. #           | Amount                    |
|--|------------------|---------------------------|
| RhoA/Rac1/Cdc42 G-LISA<br>Activation Assay Bundle<br>BK135=BK124-S+BK127-S+BK128-S | BK135            | 3 Kits<br>(24 assays/kit) |
| Cdc42 G-LISA® Activation Assay, colorimetric                                       | BK127-S<br>BK127 | 24 assays<br>96 assays    |
| Rac1,2,3 G-LISA® Activation Assay, colorimetric                                    | BK125            | 96 assays                 |
| Rac1 G-LISA® Activation Assay, colorimetric  | BK128-S<br>BK128 | 24 assays<br>96 assays    |
| Rac1 G-LISA® Activation Assay, luminescence  | BK126            | 96 assays                 |
| RalA G-LISA® Activation Assay, colorimetric  | BK129            | 96 assays                 |
| Ras G-LISA® Activation Assay, colorimetric   | BK131            | 96 assays                 |
| RhoA G-LISA® Activation Assay, colorimetric  | BK124-S<br>BK124 | 24 assays<br>96 assays    |
| RhoA G-LISA® Activation Assay, luminescence  | BK121            | 96 assays                 |
| Protease Inhibitor Cocktail (100x stock)   | PIC02            | 1 ml                      |

For isoforms not listed, see our information resources online.

## Related Activation Assay Products

## 🗲 Total RhoA ELISA

Rapidly measure Total RhoA from cell or tissue lysates using the extremely sensitive and linear Total RhoA ELISA.



## < Acti-stain Phalloidins

Acti-stain<sup>™</sup> fluorescent phalloidins provide exceptionally bright and stable probes for F-actin at an economical price.

See Pg. 15

## < Activators & Inhibitors

G-switch<sup>™</sup> small G-protein activators and inhibitors are highly potent reagents that target endogenous Rho family proteins and pathways.

See Pg. 12

## 😪 GTPase Affinity Beads & Proteins

Specifically target the active form of small G-proteins with these brightly-colored GTPase affinity beads and proteins.

See Pg. 12



# Small G-protein Tools

## Activators & Inhibitors

g

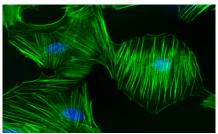


The G-switch<sup>™</sup> line of small G-protein tools are highly potent reagents that target endogenous Rho family proteins and pathways. In contrast to methods that rely on over-expression or knockdown of target proteins (e.g., DNA transfection of dominant-negative or constitutively-active Rho mutants, RNAi knockdown), G-switch<sup>™</sup> reagents act rapidly on the endogenous target protein (in minutes to hours), thereby optimizing the chance of generating a more physiologically relevant response.

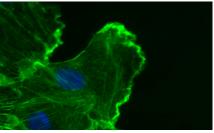
| G-protein Modulator  | Cell Entry Protein<br>Mechanism Modulatio |          | Cat. #                     | Amount                               |
|--|---|----------|----------------------------|--------------------------------------|
| Rho Activator II   | Cell                                      | Direct   | CN03-A                     | 3 x 20 μg                            |
| Deamidation of Rho Gln-63  | permeable                                 |          | CN03-B                     | 9 x 20 μg                            |
| Rho Inhibitor I<br>Specific inhibitor of Rho activity, ADP ribosylation<br>of Rho Asn-41 (very cell permeable)                     | Cell<br>permeable                         | Direct   | CT04-A<br>CT04-B<br>CT04-C | 1 x 20 μg<br>5 x 20 μg<br>20 x 20 μg |
| <b>C3 Transferase Protein</b><br>Specific inhibitor of Rho activity, ADP ribosylation<br>of Rho Asn-41 (limited cell permeability) | Pinocytosis                               | Direct   | СТ03-А<br>СТ03-С           | 1 x 25 μg<br>4 x 25 μg               |
| Rho/Rac/Cdc42 Activator I  | Cell                                      | Direct   | CN04-A                     | 3 x 20 μg                            |
| Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61   | permeable                                 |          | CN04-B                     | 9 x 20 μg                            |
| Rho Activator I  | Cell                                      | Indirect | CN01-A                     | 5 x 10 units                         |
| SHP-2 phosphatase-mediated Rho activation  | permeable                                 |          | CN01-B                     | 20 x 10 units                        |
| Rac/Cdc42 Activator II   | Receptor                                  | Indirect | CN02-A                     | 5 x 10 units                         |
| EGF receptor-mediated Rac/Cdc42 activation   | mediated                                  |          | CN02-B                     | 20 x 10 units                        |

## GEF, GAP, and GDI Effector Proteins

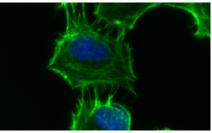
| G-protein Modulator & Effector Proteins   | Purity | Cat. #             | Amount                 |
|---|--------|--------------------|------------------------|
| <b>ARNO Protein Sec7 GEF domain protein.</b> GEF for<br>Arf1 & 6. Human recomb., 6xHis tag                      | >85%   | CS-GE07            | 3 x 20 µg              |
| Dbs His Protein, RhoGEF domain (DH/PH)<br>GEF for Cdc42 and RhoA  | >80%   | GE01-A             | 2 x 50 µg              |
| <b>p50RhoGAP GST Protein, full length</b><br>GAP for Cdc42, Rac, and Rho  | >90%   | GAP01-A<br>GAP01-B | 1 x 50 μg<br>4 x 50 μg |
| <b>p50RhoGAP GST Protein, GAP domain</b><br>GAP for Cdc42, Rac, and Rho   | >90%   | GAS01-A<br>GAS01-B | 1 x 50 μg<br>4 x 50 μg |
| Ras-GRF GEF protein Cdc25 domain<br>Human recomb., MBP tagged   | >85%   | CS-GE03            | 1 x 100 µg             |
| RhoGDI GST Protein<br>Inhibitor of Cdc42, Rac, and Rho  | >90%   | GDI01-A            | 1 x 25 µg              |
| <b>SOS1 Ras GEF Domain Protein</b><br>GEF for H-, K- or N-Ras   | >90%   | GE02<br>GE02-XL    | 1 x 100 μg<br>1 x 1 mg |
| Tiam1 GEF protein, GEF for Rac.<br>Human recomb. DHPH domain, MBP tag   | >85%   | CS-GE04            | 1 x 100 μg             |
| Vav1 GEF protein, GEF for Rac.<br>Human recomb. DHPHC1 domain Y174D mutant, 6xHis<br>tagged                     | >85%   | CS-GE05            | 1 x 100 µg             |
| <b>Vav2 GEF protein , GEF for Rac.</b><br>Human recomb. DH domain, 6xHis tagged                                 | >85%   | CS-GE06            | 1 x 100 μg             |
| <b>SOS2 exchange domain (563-1051)protein</b><br>(Human recombinant)  | >90    | CS-GE08            | 1 x 100 µg             |
| RAPGEF5 Protein: Ras association and exchange<br>domain (57– 580) wild type.<br>(Human recombinant, GST tagged) | >75%   | CS-GE09            | 1 x 100 µg             |
| <b>SOS1 Exchange Domain (564-1049) Protein</b><br>(Human Recombinant) (GST-Tagged)                              | >90%   | CS-GE10            | 1 x 100 μg             |
|   |        |                    |                        |



Stress fibers caused by Rho activation using Cat. # CN03. Actin stained green with Cat. # PHDG1.



Membrane ruffles induced by Rac activation using Cat. # CN04. Actin stained green with Cat. # PHDG1.



Microspikes induced by Cdc42 activation using Cat. # CN02. Actin stained green with Cat. # PHDG1.

## **GTPase Affinity Beads & Proteins**

| GTPase Affinity Beads and Proteins                              | Purity | Cat. #  | Amount     |
|---|--------|---------|------------|
| <b>GGA3-PBD Beads</b><br>Binds active (GTP-bound) Arf1 and Arf6 | >85%   | GGA07-A | 1 x 500 µg |
| <b>PAK-PBD Protein</b>  | >80%   | PAK01-A | 1 x 250 μg |
| Binds active (GTP-bound) Cdc42 and Rac1,2,3                     |        | PAK01-B | 4 x 250 μg |
| <b>PAK-PBD Beads</b>  | >80%   | PAK02-A | 1 x 500 μg |
| Binds active (GTP-bound) Cdc42 and Rac1,2,3                     |        | PAK02-B | 4 x 500 μg |
| <b>Raf-RBD Beads</b>  | >80%   | RF02-A  | 1 x 2 mg   |
| Binds active (GTP-bound) K-, N-, H-Ras                          |        | RF02-B  | 4 x 2 mg   |
| Rhotekin-RBD Protein<br>Binds active (GTP-bound) RhoA,B,C       | >90%   | RT01-A  | 1 x 500 µg |
| <b>Rhotekin-RBD Beads</b>                                       | >85%   | RT02-A  | 2 x 2 mg   |
| Binds active (GTP-bound) RhoA,B,C                               |        | RT02-B  | 6 x 2 mg   |



Specifically target the active form of small G-proteins with these brightly-colored GTPase affinity beads and proteins.



Small G-protein Tools (g)

#### Total RhoA ELISA Kit

Measures the total amount of RhoA in a sample of tissue or cell culture extract. Uses a sandwich ELISA to create the high specificity and sensitivity combination. 10-25  $\mu$ l sample volume. Key components included are:

- 96-well anti-Rho binding plate, contains IgY pre-coated surfaces.
- HRP detection reagents.
- Optimized sample dilution buffer.
- Primary and secondary antibodies
- RhoA control protein included.
- Comprehensive manual.

Product

**Total RhoA ELISA** 

| luded. | indicates the | it results. The plot<br>linear dependence<br>m absorbance with<br>tion of RhoA. |
|--------|---------------|---|
|        | Cat. #        | Amount  |

BK150

1.8 1.6

le 0.6 Signal

0.2 0

0

10 20 30 40

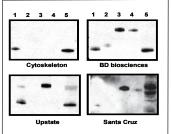
RhoA (ng/well)

96 assays

R<sup>2</sup> = 0.9914

| Measures total know levels |
|----------------------------|
|                            |
|                            |
|                            |
|                            |

#### Antibodies for Small G-proteins



Anti-Rac1 monoclonal antibody (Cat. # ARC03) does not cross-react with Rac2, 3, or Cdc42 (upper left blot), while all other commercially available Rac1 antibodies cross-react with GTPases other than Rac1.

| Small G-protein<br>Antibodies                       | Host  | Туре | Species<br>Reactivity         | Cat. #           | Amount                  |
|---|-------|------|-------------------------------|------------------|-------------------------|
| Cdc42 Specific Anti-<br>body<br>Human Cdc42 Peptide | Mouse | mAb  | Hu, Ms, Rt,<br>other extracts | ACD03<br>ACD03-S | 2 x 200 µl<br>1 x 50 µl |
| Rac1 Specific Antibody<br>Human C-terminal Peptide  | Mouse | mAb  | Hu, Ms, Rt,<br>other extracts | ARC03<br>ARC03-S | 2 x 100 μl<br>1 x 50 μl |

### Hundreds of Citations Available at Cytoskeleton.com!



| Signal Transduction Reagents   | Cat. # | Amount        |
|--|--------|---------------|
| RhoGAP Assay Biochem Kit™  | BK105  | 80-160 assays |
| <b>GTPγS</b><br>Non-hydrolyzable GTP analog, 50 μl of 20 mM                      | BS01   | 1 x 500 µg    |
| GTPase CytoPhos <sup>™</sup> Assay<br>One step assay for enzyme Kcat 0.01 to 100 | BK054  | 1000 assays   |

#### Purified G-proteins

| Purified G-proteins                                      | Purity | Cat. #                      | Amount                               |
|--|--------|-----------------------------|--------------------------------------|
| Cdc42 His Protein, constitutively-active (Q61L)          | >70%   | C6101-A                     | 1 x 10 µg                            |
| Cdc42 GST Protein, dominant-negative (T17N)              | >90%   | C17G01-A                    | 1 x 25 μg                            |
| Cdc42 GST Protein, wild-type                             | >90%   | CDG01-C                     | 8 x 25 μg                            |
| Cdc42 His Protein, wild-type                             | >90%   | CD01-A<br>CD01-C<br>CD01-XL | 1 x 100 μg<br>3 x 100 μg<br>1 x 1 mg |
| Rac1 His Protein, constitutively-active (Q61L)           | >90%   | R6101-A                     | 1 x 10 µg                            |
| Rac1 GST Protein, dominant-negative (T17N)               | >90%   | R17G01-A                    | 1 x 25 μg                            |
| Rac1 GST Protein, wild-type                              | >90%   | RCG01-C                     | 8 x 25 μg                            |
| Rac1 His Protein, wild-type                              | >90%   | RC01-A<br>RC01-C<br>RC01-XL | 1 x 100 μg<br>3 x 100 μg<br>1 x 1 mg |
| Rac2 His Protein, wild-type                              | >90%   | RC02-A                      | 1 x 100 µg                           |
| Rap1b His Protein, wild-type                             | >90%   | RR02-A                      | 1 x 100 µg                           |
| H-Ras His Protein, wild-type                             | >80%   | RS01-A<br>RS01-C            | 1 x 100 μg<br>3 x 100 μg             |
| K-Ras4B Protein, human rec., wild-type                   | >90%   | CS-RS03                     | 1 x 100 µg                           |
| K-Ras4B Protein, human rec., G12V mutant                 | >90%   | CS-RS04                     | 1 x 100 µg                           |
| N-Ras Protein, human rec., wild type                     | >90%   | CS-RS02                     | 1 x 100 µg                           |
| R-Ras Protein, human rec., wild-type                     | >90%   | CS-RS05                     | 1 x 100 µg                           |
| RhoA His Protein, constitutively-active (Q63L)           | >90%   | R6301-A                     | 1 x 10 µg                            |
| RhoA GST Protein, wild-type                              | >90%   | RHG01-C                     | 8 x 25 μg                            |
| RhoA His Protein, wild-type                              | >80%   | RH01-A<br>RH01-C<br>RH01-XL | 1 x 100 μg<br>3 x 100 μg<br>1 x 1 mg |
| RhoC His Protein, wild-type                              | >90%   | RH03-A                      | 1 x 100 µg                           |
| K-Ras4B Protein: G13D<br>(Human recombinant, 6xHis-tag)  | >90%   | CS-RS06                     | 1 x 100 µg                           |
| K-Ras4B Protein: G13S<br>(Human recombinant, 6xHis-tag)  | >85%   | CS-RS07                     | 1 x 100 µg                           |
| K-Ras4B Protein: K128A<br>(Human recombinant, 6xHis-tag) | >90%   | CS-RS08                     | 1 x 100 µg                           |
| K-Ras4B Protein: G12D<br>(Human recombinant, 6xHis-tag)  | >85%   | CS-RS13                     | 1 x 100 µg                           |
|  |        |                             |                                      |

Bulk Quantities Available email: cserve@cytoskeleton.com for bulk inquiries



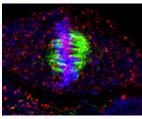
# Antibodies & Pathway Signal Detection

With Cytoskeleton's antibodies and reagents, you will benefit from several distinct advantages for your antibody-based reagents:

- All antibodies developed in house
- All antibodies manufactured in house
- · Extensive quality control that is visible to the user
- Specialist technical help

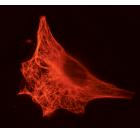
Learn More at www.cytoskeleton.com/ptm-antibodies Validation info, analysis, applications, and customer testimonials.

#### Anti-SUMO-2/3 immuno-fluorescence in mitotic cells



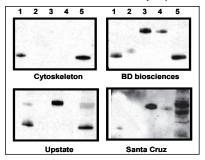
Immunofluorescence of HeLa cells in metaphase with SUMO-2/3 Antibody (Cat. # ASM23, red) and  $\alpha/\beta$ -tubulin antibody (Cat. # ATN02, green). Chromosomal DNA stained with DAPI (blue).

#### Microtubule Visualizing Antibody (Cat. # ATN02)



Microtubule network in a NIH3T3 cell illuminated with Cytoskeleton's sheep anti-tubulin antibody (ATN02). ATN02 is a pan-tubulin sheep polyclonal antibody, hence it can be co-incubated with mouse, rat or rabbit antibodies for selective dual or triple antibody staining.

#### Rac1 Antibody Specificity (Cat. # ARC03)



Western blot analysis of small G-protein versus different Rac1 antibodies. Anti-Rac1 monoclonal antibody (Cat. # ARC03) does not cross-react with Rac2, 3, or Cdc42 (upper left blot), while all other commercially available Rac1 antibodies cross-react with GTPases other than Rac1. Ln 1 - Rac1-6xHis, Ln 2- Rac2-6xHis, Ln 3 - Rac3-GST, Ln 4 -Cdc42-GST, Ln 5 - 50 µg platelet extract.

#### Small G-protein Antibodies

| Sm G-protein<br>Antibodies                            | Host  | Туре | Species<br>Reactivity         | Cat. #           | Amount                   |
|---|-------|------|-------------------------------|------------------|--------------------------|
| <b>Cdc42 Specific Antibody</b><br>Human Cdc42 Peptide | Mouse | mAb  | Hu, Ms, Rt,<br>other extracts | ACD04<br>ACD04-S | 1 x 500 μl<br>1 x 125 μl |
| Rac1 Specific Antibody<br>Human C-terminal Peptide    | Mouse | mAb  | Hu, Ms, Rt,<br>other extracts | ARC03<br>ARC03-S | 2 x 100 μl<br>1 x 25 μl  |

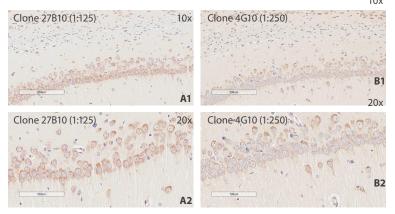
#### Cytoskeleton Protein Antibodies

| Antibodies                     | Host   | Туре | Applications       | Cat. #             | Amount                   |
|--------------------------------|--------|------|--------------------|--------------------|--------------------------|
| Anti-Pan Actin Antibody        | Mouse  | mAb  | WB, ICC            | AAN02-A<br>AAN02-S | 1 x 500 μg<br>1 x 125 μg |
| Tubulin Polyclonal<br>Antibody | Sheep  | pAb  | WB, IF, ELISA      | ATN02<br>ATN02-S   | 2 x 100 μl<br>1 x 25 μl  |
| Profilin Antibody              | Rabbit | pAb  | WB, ICC, ELISA, IP | APUF01-A           | 1 x 50 µg                |

#### New Pathway Signaling Antibodies

Cytoskeleton has expanded its offering of antibodies and reagents to study critical protein modifications. Reagents are available to study acetylation, tyrosyl phosphorylation, SUMOylation, and ubiquitination. The products are rigorously QC-tested and are particularly useful for enrichment studies of your protein of interest.

#### Immunohistochemical analysis of rat neuronal tissue: Anti-phosphotyrosine mAb 27B10 (Cat. # APY03) vs. $4G10_{10x}$



Anti-phosphotyrosine staining in rat neuronal tissue with Cytoskeleton's monoclonal antibody 27B10 (Cat. # APY03: A1, A2) vs. monoclonal antibody 4G10 (B1, B2). Proteinase K antigen retrieval used. Note the stronger and more specific anti-phosphotyrosine staining with Cytoskeleton's antibody 27B10 versus 4G10 antibody.

#### Pathway Signaling Antibodies

| PTMtrue Antibody  | Host  | Туре | Applications         | Cat. #                       | Amount                                |
|---|-------|------|----------------------|------------------------------|---------------------------------------|
| Acetyl Lysine Antibody  | Mouse | mAb  | WB, IF, IP, CHiP     | AAC01<br>AAC01-S             | 2 x 100 μl<br>1 x 25 μl               |
| Acetyl Lysine Affinity Beads  | Mouse | mAB  | IP                   | AAC04-beads                  | 4 x 500 μl                            |
| Phosphotyrosine Antibody  | Mouse | mAb  | WB, IP, IF,<br>ELISA | APY03<br>APY03-S             | 2 x 100 μl<br>1 x 25 μl               |
| Anti-Phosphotyrosine<br>Affinity Beads                                      | Mouse | mAb  | IP                   | APY03-Beads                  | 4 x 300 µl                            |
| Phosphotyrosine Antibody<br>(HRP conjugate)                                 | Mouse | mAb  | WB                   | APY03-HRP<br>APY03-HRP-S     | 1 x 100 μl<br>1 x 25 μl               |
| SUMO-2/3 Antibody<br>(Clone 12F3)   | Mouse | mAb  | WB, IF, IP           | ASM23<br>ASM23-S             | 2 x 100 μl<br>1 x 25 μl               |
| SUMO-2/3 Antibody<br>(Clone 11G2)   | Mouse | mAb  | IF, IP               | ASM24<br>ASM24-S             | 2 x 200 μl<br>1 x 150 μl              |
| SUMO-2/3<br>Affinity Beads  | Mouse | mAb  | IP                   | ASM24-Beads                  | 2 x 400 µl                            |
| Ubiquitin Antibody  | Mouse | mAb  | WB, IF               | AUB01<br>AUB01-S<br>AUB01-XL | 2 x 100 μl<br>1 x 25 μl<br>4 x 500 μl |
| Ubiquitin Affinity Beads<br>(binds mono-/poly-ubiquitin<br>tagged proteins) | n/a   | n/a  | IP                   | UBA01-beads                  | 2 x 120 μl                            |
| Control for Ippt IgG Beads  | n/a   | n/a  | IP                   | CIG01-beads                  | 10 assays                             |
| Control beads for<br>Acetylation Ippt                                       | n/a   | n/a  | IP                   | CIG02-beads                  | 10 assays                             |
| Control beads for SUMO1 or 2/3 lppt   | n/a   | n/a  | IP                   | CIG03-beads                  | 10 assays                             |
| Control for Ubiquitin Affinity<br>Beads                                     | n/a   | n/a  | IP                   | CUB02-beads                  | 10 assays                             |
| SUMO-1 Antibody<br>(Clone 5D8B16)   | Mouse | mAb  | WB, IP               | ASM01<br>ASM01-S             | 1 x 100 μl<br>1 x 25 μl               |



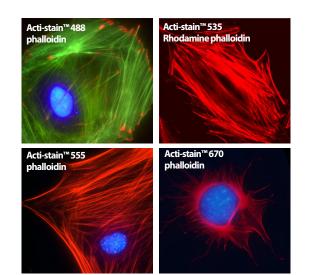
# **Actin Visualization**



#### Acti-stain<sup>™</sup> Fluorescent Phalloidins and Spirochrome<sup>™</sup> Live Cell Probes

The Acti-stain<sup>™</sup> line of fluorescent phalloidins has been developed with an emphasis on creating exceptionally bright and stable probes for F-actin offered at an economical price. Side-by-side comparisons with similar products insure considerable savings without sacrificing quality when switching to an Acti-stain<sup>™</sup> probe. The combination of in-house manufacturing, stringent quality control, and convenient packaging provides a great value. Give them a try and see for yourself.

For more information, citations and comparison to other fluorescent phalloidins, visit: cytoskeleton.com/actin/acti-stain



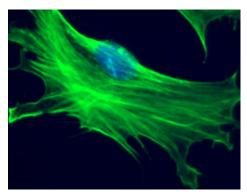
Swiss 3T3 cells stained with Acti-stain<sup>™</sup> Fluorescent Phalloidins

| Product   | Excitation | Emission | Signal stability without antifade*<br>(Tı/2 in secs) | Cat. #   | Amount**   |
|---|------------|----------|--|----------|------------|
| Acti-stain™ 488 phalloidin                                    | 480 nm     | 535 nm   | 57   | PHDG1-A  | 300 Slides |
| Acti-stain <sup>™</sup> 535 phalloidin (Rhodamine phalloidin) | 535 nm     | 585 nm   | 27   | PHDR1    | 300 Slides |
| Acti-stain™ 555 phalloidin                                    | 535 nm     | 585 nm   | 46   | PHDH1-A  | 300 Slides |
| Acti-stain™ 670 phalloidin                                    | 640 nm     | 670 nm   | 18   | PHDN1-A  | 300 Slides |
| SiR-Actin Kit<br>Includes SiR700-Actin and Verapamil          | 630 nm     | 680 nm   | na***  | CY-SC001 | 50 nmol    |
| SPY555-FastAct™   | 555 nm     | 580 nm   | na***  | CY-SC205 | 100 Slides |
| SPY650-FastAct™   | 652 nm     | 674 nm   | na***  | CY-SC505 | 100 Slides |

\* Stability measured with stained slides without antifade. For comparison, fluorescein phalloidin has a T<sub>1/2</sub> of 6 secs.
\*\* One slide equals enough phalloidin to stain a 25 mm<sup>2</sup> coverslip.

#### Pan-Actin Antibody

AAN02 is a mouse monoclonal antibody against actin protein. The antibody has been shown to recognize  $\alpha$ -skeletal,  $\alpha$ -cardiac,  $\alpha$ -smooth muscle,  $\beta$ -cytoplasmic,  $\gamma$ -smooth muscle and  $\gamma$ - cytoplasmic actin isotypes.



Immunofluorescence images of mouse Swiss 3T3 cells stained with antiactin antibody. Swiss 3T3 cells were grown to 25% confluency on poly-lysine and laminin coverslips. 3T3 cells were fixed with PFA. Cells were permeabilized with methanol followed by 0.5% Triton X-100 as described in the method. IF staining using 1:500 dilution of anti-actin antibody in PBS is shown (areen).

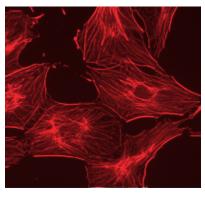
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| Product   | Cat. #  | Amou    |
|---|---------|---------|
| Anti-Pan Actin Mouse Monoclonal Antibody (Clone<br>7A8.2.1) | AAN02-S | 1 x 125 |
| Anti-Pan Actin Mouse Monoclonal Antibody (Clone<br>7A8.2.1) | AAN02   | 1 x 500 |
|   |         |         |

\*\*\* SiR was approximately ten fold more stable than Alexa647 and as stable as atto647N (Lukinavičius, et. al.; Nature Chemistry, 5, 132–139, 2013.). SiR-Actin is a trademarks of Spirochrome SA (Switzerland).

#### F-actin Visualization Biochem Kit<sup>®</sup>

Fix and permeabilize tissue culture cells while preserving structure of the F-actin cytoskeleton. Subsequently, the F-actin cytoskeleton is stained with fluorescent (rhodamine) phalloidin (Cat. # PHDR1) that is provided in the kit.



The F-actin cytoskeleton of Swiss 3T3 cells visualized with rhodamine phalloidin and using fixatives and cell permeabilizing reagents from the F-actin Visualization Biochem Kit™.

cytoskeleton.com

| Product                            | Cat. # | Amount     |
|------------------------------------|--------|------------|
| F-actin Visualization Biochem Kit™ | BK005  | 300 assays |

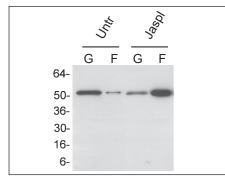
# Actin Biochem Kits

### G-actin/F-actin In Vivo Assay Biochem Kit<sup>™</sup>

- Quantitates monomeric vs polymeric actin in cell/tissue lysates
- Reproducible and accurate method
- Contains all needed reagents

Lyse cells or tissue in the F-actin stabilizing buffer, preserving the G-actin:F-actin ratio. Centrifuge samples, separating supernatants (G-actin) and pellets (F-actin) which are then run on a gel for Western blot analysis.

## Reorganization of actin after treatment with jasplakinolide



Swiss 3T3 cells were treated with jasplakinolide (Jaspl) or left untreated (Untr) and the G-actin (G) and F-actin (F) content was assayed using the G-actin/F-actin kit. Treatment with jasplakinolide resulted in a potent accumulation of F-actin.

| Product  | Cat. # | Amount           |
|--|--------|------------------|
| G-actin/F-actin <i>In Vivo</i><br>Assay Biochem Kit™ | BK037  | 30-100<br>assays |
| Protease Inhibitor Cocktail<br>(100x solution)       | PIC02  | 1 ml             |

#### The role of actin oxidation

A seminal study by the Terman group identified a role for the enzyme, MICAL, in mediating oxidation of Met44 and Met47 of actin *in vitro*<sup>5</sup>.

These in vitro studies showed that MetO at Met44 was sufficient to promote both severing of filaments and decreased polymerization.

Cytoskeleton is pleased to offer the modified versions of actin and pyrene actin for biochemical studies.

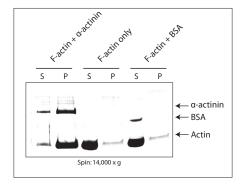
#### 16 cytoskeleton.com

## Actin Binding Protein Spin-Down Assay Biochem Kit

- Identifies and characterizes Actin Binding Proteins (ABPs)
- Generation of saturation binding curves
- Muscle (BK001) or non-muscle (BK013) actin

This co-sedimentation assay will help you identify whether your ABP is a F-actin binding protein, a F-actin severing protein, has F-actin bundling activity, or is a G-actin binding protein.

#### Actin bundling assay using kit BK001



**F-actin was incubated alone or together with**  $\alpha$ **-actinin or BSA.** Bundled F-actin was pelleted by a 14,000 x g centrifugation and pellets (P) and supernatants (S) were run on a SDS-PAGE gel. Only in the presence of the F-actin bundling protein  $\alpha$ -actinin is actin pelleted at this centrifugation speed.

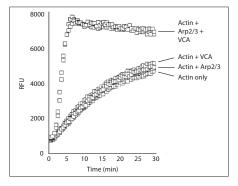
| Product   | Cat. # | Amount           |
|---|--------|------------------|
| Actin Binding Protein Spin-<br>Down Assay Biochem Kit"<br>(skeletal muscle actin)       | BK001  | 30-100<br>assays |
| Actin Binding Protein<br>Spin-Down Assay Biochem Kit <sup>™</sup><br>(non-muscle actin) | BK013  | 30-100<br>assays |

### Actin Polymerization Assay Biochem Kit<sup>™</sup>

- Utilizes fluorescent pyrene-actin
- F-actin polymerization and depolymerization
- Works with multiple sources of actin
- Valuable for characterizing ABPs

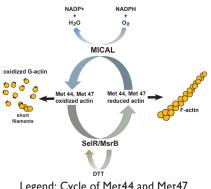
This kit is based upon the enhanced fluorescence of pyrene-conjugated actin that occurs during polymerization. Its versatility allows the study of the effects on polymerization (or depolymerization) of a compound, tissue extract, or protein of interest.

#### Characterization of ABPs using Actin Polymerization Biochem Kit™



Effects of Arp2/3 (Cat. # RP01P) and the WASP VCA (Cat. # VCG03) domain on actin polymerization *in vitro*. Arp2/3 or the WASP VCA domain alone has little effect on the rate of actin polymerization, while the combination of the two leads to an activation of the actin nucleating Arp2/3 complex and a subsequent increased rate of actin polymerization.

| Product                                    | Cat. # | Amount           |
|--|--------|------------------|
| Actin Polymerization<br>Assay Biochem Kit™ | BK003  | 30-100<br>assays |



#### Legend; Cycle of Met44 and Met47 oxidation and reduction by catalytic activity of Mical and MsrB enzymes.

#### **MICAL-Oxidized Actin Products**

| Description                               | Amount                | Cat. #            |
|---|-----------------------|-------------------|
| MICAL-Oxidized (Pyrene labeled) Actin     | 2 x 250 ug            | MPAX1             |
| Protein (95% pure) Rabbit Skeletal Muscle | 1 x 1 mg              | MPAX1-XL          |
| MICAL-Oxidized Actin Protein(>95% pure)   | 2 x 250 ug            | MXA95             |
| Rabbit Skeletal Muscle                    | 1 x 1 mg              | MXA95-XL          |
| MICAL-1 Protein 6xHis                     | 2 x 50 ug<br>1 x 1 mg | MIC01<br>MIC01-XL |
| MsrB2 Protein 6xHis                       | 2 x 50 ug<br>1 x 1 mg | MB201<br>MB201-XL |
| Actin Protein (pyrene labeled)            | 1 x 1 mg              | AP05-A            |
| Rabbit Skeletal Muscle                    | 5 x 1 mg              | AP05-B            |
| Actin Protein (>95% pure)                 | 1 x 1 mg              | AKL95-B           |
| Rabbit Skeletal Muscle                    | 5 x 1 mg              | AKL95-C           |

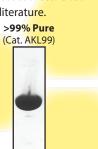
# Actin & ECM Proteins



## High Purity

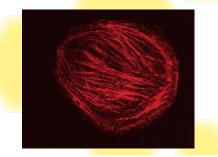
The highest purity actin available. Purities greater than 99% from most sources. Cited hundreds of times in the literature.





### Labeled Actins

Highly pure, biologically active actins labeled with Rhodamine, Pyrene, or Biotin.



## Unlabeled Actin Proteins

| Unlabeled Actins                                    | Source                            | Purity | Cat. #  | Amount   |
|---|-----------------------------------|--------|---|--|
| Actin Protein                                       | Rabbit skeletal<br>muscle         | >99%   | AKL99-A<br>AKL99-B<br>AKL99-C<br>AKL99-D<br>AKL99-E | 4 x 250 μg<br>2 x 1 mg<br>5 x 1 mg<br>10 x 1 mg<br>20 x 1 mg |
| Actin Protein                                       | Rabbit skeletal<br>muscle         | >97%   | AKL95-B<br>AKL95-C                                  | 1 x 1 mg<br>5 x 1 mg   |
| Actin Protein                                       | Bovine cardiac<br>muscle          | >99%   | AD99-A<br>AD99-B                                    | 1 x 1 mg<br>5 x 1 mg   |
| Actin Protein                                       | Smooth muscle,<br>chicken gizzard | >99%   | AS99-A<br>AS99-B                                    | 1 x 1 mg<br>5 x 1 mg   |
| Actin Protein                                       | Human plate-<br>let, non-muscle   | >99%   | APHL99-A<br>APHL99-C<br>APHL99-E                    | 2 x 250 μg<br>1 x 1 mg<br>5 x 1 mg                           |
| Pre-formed Actin Filaments                          | Rabbit skeletal<br>muscle         | >99%   | AKF99-A<br>AKF99-B                                  | 1 x 1 mg<br>5 x 1 mg   |
| Actin Thin Filament<br>(Ca2+ sensitive complex)     | Bovine cardiac<br>muscle          | 90%    | TFC01   | 1 x 1 mg   |
| Actin Thin Filament<br>(Ca2+ sensitive complex)     | Rabbit skeletal<br>muscle         | 90%    | CS-TFC02  | 1 x 1 mg   |
| Ebashi Complex (complex of tromyosin/tropomodulin ) | Bovine cardiac<br>muscle          | 70%    | CS-TT05   | 1 x 1 mg   |

## Labeled Actin Proteins

| Labeled Actins                | Source                        | Purity | Cat. #           | Amount                  |
|-------------------------------|-------------------------------|--------|------------------|-------------------------|
| Biotinylated Actin<br>Protein | Rabbit skeletal<br>muscle     | >99%   | AB07-A<br>AB07-C | 5 x 20 μg<br>20 x 20 μg |
| Pyrene Actin Protein          | Rabbit skeletal<br>muscle     | >99%   | AP05-A<br>AP05-B | 1 x 1 mg<br>5 x 1 mg    |
| Pyrene Actin Protein          | Bovine cardiac<br>muscle      | >99%   | CS-AP07          | 1 x 250 µg              |
| Rhodamine Actin<br>Protein    | Human platelet,<br>non-muscle | >99%   | APHR-A<br>APHR-C | 4 x 10 μg<br>20 x 10 μg |

## Actin Antibodies

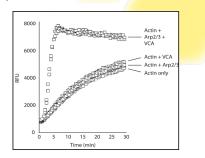
| Antibodies                    | Antigen                    | Host   | Grade                | Cat. #             | Amount                   |
|-------------------------------|----------------------------|--------|----------------------|--------------------|--------------------------|
| Anti-Pan<br>Actin<br>Antibody | Purified Actin             | Mouse  | Affinity<br>Purified | AAN02-A<br>AAN02-S | 1 x 500 μg<br>1 x 125 μg |
| Profilin Antibody             | Purified human<br>profilin | Rabbit | Affinity<br>Purified | APUF01-A           | 1 x 50 µg                |

## Actin Buffers

| Actin Buffers  | Cat. #                 | Amount                  |
|--|------------------------|-------------------------|
| General Actin Buffer (10 ml or 100 ml when resuspended)<br>For resuspending & diluting G-actin protein | BSA01-001<br>BSA01-010 | 1 x 10 ml<br>1 x 100 ml |
| Actin Polymerization Buffer (10X stock when resuspended)<br>For the polymerization of actin            | BSA02-001              | 1 x 2 ml                |
| ATP (100 mM stock solution when resuspended)<br>ATP is required for actin stability and polymerization | BSA04-001              | 1 x 1 ml                |

## **Biologically Active**

Actin polymerization stimulated by Arp2/3 complex and the VCA domain of WASP measured by Pyrene Actin fluorescence (Cat.# AP05).



## Actin Binding Proteins

| Actin Binding Proteins                                 | Source   | Purity | Cat. #                       | Amount                               |
|--|--|--------|------------------------------|--------------------------------------|
| $\alpha$ -Actinin Protein                              | Rabbit skeletal muscle   | >90%   | AT01-A<br>AT01-C             | 2 x 50 μg<br>10 x 50 μg              |
| Arp2/3 Protein Complex                                 | Porcine brain  | >90%   | RP01P-A<br>RP01P-B           | 2 x 50 μg<br>6 x 50 μg               |
| Cofilin Protein  | Recombinant human<br>cofilin 1   | 95%    | CF01-A<br>CF01-C             | 1 x 100 μg<br>4 x 100 μg             |
| Gelsolin Protein                                       | Recombinant human,<br>plasma isoform   | >95%   | HPG6-A<br>HPG6-B             | 4 x 20 μg<br>20 x 20 μg              |
| Lysyl Oxidase Protein                                  | Bovine leg tendon  | >80%   | CS-LXE01                     | 1 x 5 µg                             |
| Myosin II Cardiac Protein                              | Bovine cardiac muscle  | 95%    | MY03-A<br>MY03-B             | 5 x 1 mg<br>20 x 1 mg                |
| S1 Myosin Protein                                      | Rabbit skeletal muscle<br>Chymotrypsin digest of<br>Cat. # MY02 plus chromat | >90%   | CS-MYS04                     | 1 x 250 μg                           |
| S1 Myosin Protein                                      | Bovine cardiac muscle<br>Chymotrypsin digest of<br>Cat. # MY03 plus chromat. | >90%   | CS-MYS03                     | 1 x 250 µg                           |
| Heavy Meromyosin Protein                               | Bovine cardiac muscle<br>Chymotrypsin digest of<br>Cat. # MY03 plus FPLC.    | 90%    | CS-MH03                      | 1 x 100 µg                           |
| Myosin II Protein                                      | Rabbit skeletal muscle   | 95%    | MY02-A<br>MY02-B             | 5 x 1 mg<br>20 x 1 mg                |
| Heavy Meromyosin Protein                               | Rabbit skeletal muscle<br>Chymotrypsin digest of<br>Cat. # MY02.             | 90%    | MH01-A                       | 4 x 50 μg                            |
| Profilin Protein                                       | Recombinant human<br>profilin 1  | >95%   | PR02-A<br>PR02-B<br>PR02-XL2 | 1 x 100 μg<br>1 x 500 μg<br>1 x 1 mg |
| WASP protein VCA Domain:<br>Activates Arp2/3, GST tag. | Recombinant human  | >95%   | VCG03-A                      | 1 x 500 μg                           |

#### Labeled ECM Proteins

| Labeled ECMs   | Source                                | Purity | Cat. #             | Amount                  |
|--|---------------------------------------|--------|--------------------|-------------------------|
| Fibronectin<br>Red fluorescent, rhodamine                              | Bovine serum                          | >80%   | FNR01-A<br>FNR01-B | 5 x 20 μg<br>20 x 20 μg |
| <b>Fibronectin</b><br>Green fluorescent, HiLyte Fluor <sup>™</sup> 488 | Bovine serum                          | >80%   | FNR02-A<br>FNR02-B | 5 x 20 μg<br>20 x 20 μg |
| <b>Fibronectin</b><br>Biotinylated                                     | Bovine serum                          | >80%   | FNR03-A<br>FNR03-B | 5 x 20 μg<br>20 x 20 μg |
| <b>Laminin</b><br>Red fluorescent, rhodamine                           | Engelbreth-Holm-<br>Swarm mouse tumor | >90%   | LMN01-A<br>LMN01-B | 5 x 20 μg<br>20 x 20 μg |
| <b>Laminin</b><br>Green fluorescent, HiLyte Fluor <sup>™</sup> 488     | Engelbreth-Holm-<br>Swarm mouse tumor | >90%   | LMN02-A<br>LMN02-B | 5 x 20 μg<br>20 x 20 μg |
| <b>Laminin</b><br>Biotinylated   | Engelbreth-Holm-<br>Swarm mouse tumor | >90%   | LMN03-A<br>LMN03-B | 5 x 20 μg<br>20 x 20 μg |

HiLyte Fluor is a trademark of Anaspec, Inc. (CA).

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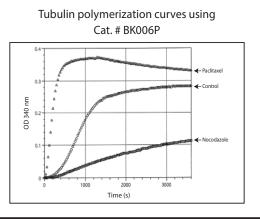
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## Tubulin Polymerization Assays

Tubulin polymerization assays are available in two formats: 1) the light scatter (also called absorbance or turbidometric) and 2) the fluorescence format based on the DAPI fluorophore. Both methods are sensitive to inhibitors and enhancers of polymerization. BK004P is an absorbance-based format used for hit or no hit screening results, whereas BK006P is for IC50 determinations which need more accuracy. BK011P, the fluorescent-based format, is used for screening and IC50s and is the most economical per assay.

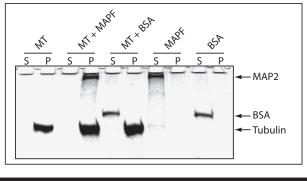


| Product  | Cat. # | Amount          |
|--|--------|-----------------|
| <b>Tubulin Polymerization Assay Biochem Kit™</b><br>Turbidometric-based, >99% pure tubulin | BK006P | 24-30<br>assays |
| Tubulin Polymerization Assay Biochem Kit™<br>Turbidometric-based, >97% pure tubulin        | BK004P | 24-30<br>assays |
| Tubulin Polymerization Assay Biochem Kit™<br>Fluorescence-based, >99% pure tubulin         | BK011P | 96<br>assays    |

#### **Tubulin Binding Assays**

The Microtubule Binding Assay provides a robust method to identify and quantify how your test substance interacts with microtubules (see below). Biotinylated tubulin (Cat. # T333P) for use in subunit (heterodimer) binding assays is also available. See the SPA-based ligand competition assay described by Tahir et al. 2000 (Biotechniques, v29, pp156-160.).

## Microtubule Binding Assay (Cat. # BK029) used to detect MAP binding to microtubules



| Product   | Cat. #                         | Amount                                |
|---|--------------------------------|---------------------------------------|
| Tubulin (biotin labeled)                                    | T333P-A<br>T333P-B<br>T333P-XL | 5 x 20 μg<br>20 x 20 μg<br>1 x 500 μg |
| Microtubule Binding Protein Spin-Down Assay<br>Biochem Kit™ | BK029                          | 30-100<br>assays                      |

## Tubulin Buffers, Reagents, & MAPs

| Tubulin Buffers, Reagents, & MAPs  | Cat. #                 | Amount                    |
|--|------------------------|---------------------------|
| General Tubulin Buffer<br>10 ml or 100 ml when resuspended                           | BST01-001<br>BST01-010 | 1 x 10 ml<br>1 x 100 ml   |
| GTP (100 mM stock when resuspended)  | BST06-001<br>BST06-010 | 1 x 100 μl<br>10 x 100 μl |
| Tubulin Glycerol Buffer<br>Enhances tubulin polymerization                           | BST05-001              | 1 x 10 ml                 |
| Microtubule-Associated Protein (MAP) Fraction<br>Bovine brain MAP fraction, 70% MAP2 | MAPF-A<br>MAPF-C       | 1 x 100 μg<br>5 x 100 μg  |
| Paclitaxel (2 mM stock when resuspended)<br>Stabilizes microtubules                  | TXD01                  | 10 x 100 µl               |
| <b>Tau Protein</b><br>Bovine brain   | TA01-A<br>TA01-B       | 1 x 50 μg<br>3 x 50 μg    |

#### **Specialized Tubulins**

These specialized tubulins help exploit the diversity between host and pathogen tubulin isotypes. In combination with these proteins, micro-assays provide the most economical method of measuring drug interaction.



|     | Products   | Cat. #   | Amount     |
|-----|--|----------|------------|
|     | Caki-1 Tumor Tubulin Protein   | CS-TM001 | 1 x 250 µg |
| NEW | Alpha Tubulin N-Acetyltransferase 1<br>(2-236) Protein (Human Recombinant) | CS-TAT01 | 1 x 100 µg |
| NEW | Acetylated Tubulin protein<br>Source: Porcine Brain                        | CS-TAC01 | 1 x 500 µg |



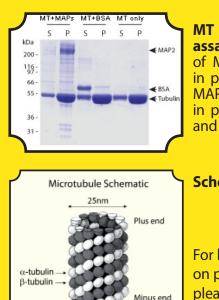
# Tubulin & FtsZ Proteins



#### Pre-formed Microtubules (Cat.# MT002)

- Substrate for discovery and characterization of microtubule binding proteins
- Determine IC50s for kinesin inhibitors
- Substrate for kinesin ATPases
- Ideal for HTS applications

Microtubules serve as a substrate for kinesin motor proteins. Kinesin motor proteins orchestrate a wide range of kinetic events within a cell. They have been shown to move cargoes such as chromosomes and vesicles along Microtubule tracks. Cytoskeleton's pre-formed Microtubules are an excellent substrate for detecting Microtubule Binding Proteins in combination with the Kinesin Enzyme Linked Inorganic Phosphate ATPase Kit (Cat. #BK060).



**MT binding spin-down assay using MT002.** >80% of MT002 (arrow: Tubulin) is in pellet (P) after spin-down. MAPs bind to MTs and end up in pellet while BSA does not and stays in supernatant (S).

#### **Schematic of a Microtubule**

For large volume discounts on pre-formed microtubules please email us at: tservice@cytoskeleton.com

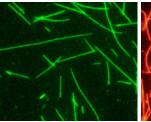
#### Unlabeled Tubulin Proteins

| Unlabeled Proteins  | Source                   | Purity                  | Cat. #                                | Amount   |
|---|--------------------------|-------------------------|---------------------------------------|--|
| <b>Tubulin Protein</b><br>Lyophilized (no glycerol)           | Porcine<br>Brain         | >99%                    | T240-A<br>T240-B<br>T240-C<br>T240-DX | 1 x 1 mg<br>5 x 1 mg<br>20 x 1 mg<br>1 x 10 mg |
| <b>Tubulin Protein, MAP rich</b><br>Lyophilized (no glycerol) | Porcine<br>Brain         | 70% tubulin<br>30% MAPs | ML116-A<br>ML116-B<br>ML116-DX        | 1 x 1 mg<br>5 x 1 mg<br>1 x 10 mg              |
| Tubulin for HTS Applications                                  | Porcine<br>Brain         | 97%                     | HTS03-A<br>HTS03-B                    | 1 x 4 mg<br>1 x 40 mg                          |
| <b>Tubulin Protein</b><br>Frozen (no glycerol)                | Porcine<br>Brain         | >99%                    | T238P-A<br>T238P-B<br>T238P-C         | 1 x 1 mg<br>5 x 1 mg<br>20 x 1 mg              |
| <b>Microtubules</b><br>pre-formed, lyophilized                | Porcine<br>brain         | >99%                    | MT002-A<br>MT002-XL                   | 4 x 500 μg<br>1 x 10 mg                        |
| Caki-1 Tumor Tubulin Protein                                  | Caki-1 Tu-<br>mor Tissue | >90%                    | CS-TM001                              | 1 x 250 µg                                     |

## More Tubulin Biochem Kits<sup>™</sup> & Antibodies

| Tubulin Biocher  | Cat. #   | Amount |                    |                      |  |  |
|--|--|--------|--------------------|----------------------|--|--|
| <b>Microtubule / Tu</b><br>Quantitates <i>in vivo</i> r  |  | BK038  | 30-100<br>assays   |                      |  |  |
| Tubulin polyclonal antibody (host: sheep)         ATN02         2 x 100 µl           Detects all species and isoforms of tubulin         ATN02-S         1 x 25 µl |  |        |                    |                      |  |  |
| FtsZ Proteins  |  |        |                    |                      |  |  |
| FtsZ Proteins  | Source   | Purity | Cat. #             | Amount               |  |  |
| FtsZ Protein   | <i>S. aureus</i> , recombinant,<br>6xHis-tagged  | >90%   | FTZ02-A<br>FTZ02-B | 1 x 1 mg<br>5 x 1 mg |  |  |
| FtsZ Protein   | S. pneumoniae,<br>recombinant, 6xHis-tagged      | >90%   | FTZ03-A<br>FTZ03-B | 1 x 1 mg<br>5 x 1 mg |  |  |
| FtsZ Protein   | <i>E. faecalis,</i> recombinant,<br>6xHis-tagged | >90%   | FTZ04-A<br>FTZ04-B | 1 x 1 mg<br>5 x 1 mg |  |  |
| FtsZ Protein   | <i>E. coli,</i> recombinant,<br>6xHis-tagged     | >90%   | FTZ05-A<br>FTZ05-B | 1 x 1 mg<br>5 x 1 mg |  |  |

#### Labeled Tubulin Proteins

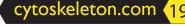


HiLyte Fluor™ 488 Labeled Tubulin - Cat. # TL488M HiLyte Fluoris a trademark of Anaspec, Inc. (CA).



TRITC Rhodamine Labeled Tubulin - Cat. # TL590M

| Labeled Tubulin Proteins             | Ex / Em<br>wavelength          | T <sub>1/2</sub> of<br>fluorescene (s) | Source           | Purity | Cat. #                         | Amount                                |
|--------------------------------------|--------------------------------|--|------------------|--------|--------------------------------|---------------------------------------|
| AMCA Labeled Tubulin                 | 350 +/-20 nm<br>440 +/-20 nm   | 10                                     | Porcine<br>Brain | >99%   | TL440M-A<br>TL440M-B           | 5 x 20 μg<br>20 x 20 μg               |
| HiLyte Fluor™ 488<br>Labeled Tubulin | 460 +/-20 nm<br>520 +/-20 nm   | 300                                    | Porcine<br>Brain | >99%   | TL488M-A<br>TL488M-B           | 5 x 20 μg<br>20 x 20 μg               |
| TRITC Rhodamine<br>Labeled Tubulin   | 535 +/-20 nm<br>590 +/-20 nm   | 50                                     | Porcine<br>Brain | >99%   | TL590M-A<br>TL590M-B           | 5 x 20 μg<br>20 x 20 μg               |
| X-Rhodamine<br>Labeled Tubulin       | 560 +/- 20 nm<br>620 +/- 20 nm | 70                                     | Bovine<br>Brain  | >99%   | TL620M-A<br>TL620M-B           | 5 x 20 μg<br>20 x 20 μg               |
| HiLyte Fluor™ 647<br>Labeled Tubulin | 620 +/-20 nm<br>670 +/-20 nm   | 80                                     | Porcine<br>Brain | >99%   | TL670M-A<br>TL670M-B           | 5 x 20 μg<br>20 x 20 μg               |
| Biotin Tubulin                       | na                             | na                                     | Porcine<br>Brain | >99%   | T333P-A<br>T333P-B<br>T333P-XI | 5 x 20 μg<br>20 x 20 μg<br>1 x 500 μg |



#### Ordering information for USA:

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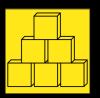
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