Activators of Rho, Rac and Cdc42

Examples of known RhoA activators

Activator*	Treatment	Cell Line	Response	Type of Assay	Citation
Lysophosphatidylcholine (lysophospho-lipid)	100 nM	human melanocytes	After 1 min, 1.5 fold increase over control. Ac-tivation peaked at 5 min (5.3 fold increase) and declined to baseline by 60 min	Rho G-LISA (Cat. # BK124)	Scott et al., 2007. J Invest Dermatol. 127, 668
Calpeptin (Cat. # CN01)	0.5 mU/mL	uterine myocytes	After 15 min treatment, 2.4 fold increase over vehicle control	RhoA G-LISA (Cat. # BK124)	Aguilar et al., 2011. PLoS ONE 6(6): e20903. doi:10.1371/journal.pone.0020903
Colchicine (microtubule destabilizer)	10 µg/ml	Swiss 3T3 cells, adherent or suspension	Maximal activation of 2-4 fold activation after 30 min	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578
Nocodazole (microtubule destabilizer)	10 µM	MG63 human osteosarcoma cells & HeLa cells	Maximal activation of 2-3 fold activation after 30 min	Actin morphology, rhotekin-RBD pulldown	Zhang et al., 1997 . Mol Biol Cell. 8, 1415 Maddox and Burridge, 2003. J Cell Biol. 160, 255
Vinblastine (microtubule destabilizer)	50 µM	MG63 human osteosarcoma cells	Maximal activation of 2-4 fold activation after 30 min	Actin morphology	Zhang et al., 1997 . Mol Biol Cell. 8, 1415
Cytochalasin D (actin filament desta-bilizer)	0.5 µg/ml	Swiss 3T3 cells, adherent or suspension	Maximal activation of 1-2 fold after 60 min	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578
Sphingosine -1-phosphate (serum lipid & GPCR agonist)	1 µg/ml	Swiss 3T3 cells, adherent or suspension	Maximal activation of 1-2 fold after 2 min for 3T3 cells and 20 min for HUVEC cells	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578 Vouret-Craviari et al., 2002. J Cell Sci. 115, 2475
Serum	5 - 10%	Swiss 3T3 cells, adherent or suspension	Maximal activation of 2-6 fold (10%) and 1-2 fold (5%) after 1-5 min	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578
Lysophosphatidic acid (LPA) (serum lipid & GPCR agonist)	1 µg/ml	Swiss 3T3 cells, adherent or suspension	Maximal activation of 2-6 fold after 1 min then dropping to basal after 30 min	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578
Lysophosphatidic acid (LPA) (serum lipid & GPCR agonist)	1 µM	N1E-115 neuronal cells	Maximal activation of 3-5 fold after 3 min	Rho-kinase pull down assay	Kranenburg et al., 1999. Mol Biol Cell. 10, 1851
Fibronectin (extracellular matrix protein)	Culture plate is coated with fibronectin	Swiss 3T3 cells	Biphasic regulation after plating cells on fibronection coated plates. Initial period of low RhoA activity (10-20 min) followed by a 1-7 fold activation peaking at 60-90 minutes and then dropping to basal levels after 6 h	Rhotekin-RBD pulldown	Ren et al., 1999. EMBO J. 18, 578
Bombesin	10 nM	Swiss 3T3 cells	Maximal activation of 2-3 fold after 1 min which is sustained for at least 30 min	Actin morphology	Ridley and Hall, 1992. Cell. 70, 389

Activators of Rho, Rac and Cdc42

Examples of known Rac activators

Activator*	Treatment	Cell Line	Response	Type of Assay	Citation
Epidermal Growth Factor (Cat. # CN02)	10 ng/ml	Swiss 3T3 cells	Maximal activation after 2 min	Rac G-LISA (Cat. # BK126 or BK128)	In-house QC results
Epidermal Growth Factor	50 ng/ml	U87MG human glioblastoma cells	Maximal activation at 5 min. With 2D cultures, 55% increase over control and with 3D cultures, 25% over control	Rac G-LISA	Kim et al., 2008. Mol Biol Cell. 19, 4249
MCP-1 (chemokine and ligand for GPCR CCR2)	10 ng/ml	murine alveolar macrophages; J774 macrophages murine peritoneal macrophages	maximal activation with 4 h treatment	Rac G-LISA; PAK-PBD pulldown	Tanaka et al., 2010. Biochem Biophys Res Comm. 399, 677
heregulin beta 1 (ligand for the ErbB3/ ErbB4 tyrosine kinase-coupled recep-tors)	0-30 ng/ml; used 10 ng/ml for time course	breast cancer cell lines MCF-7 and T-47D serum-starved for 48 h	dose-dependent effect with activation at 2 min, maximal by 10 min (MCF) or 5 min (T-47D)	PAK-PBD pulldown	Yang et al., 2006. Molec and Cellular Biol. 26, 831
Epidermal Growth Factor	100 ng/ml	breast cancer cell lines MCF-7 and T-47D serum-starved for 48 h	maximal activation by 2 min	PAK-PBD pulldown	Yang et al., 2006. Molec and Cellular Biol. 26, 831
hyperosmotic exposure	100 mM NaCl added into the isotonic medium for 10 min	neutrophils	2.5 fold increase over control	PAK-PBD pulldown	Lewis et al., 2002. Am J Physiol Cell. Physiol. 282, C271
hyperosmotic exposure	200 mM sucrose added, a non-ionic osmolyte, for 10 min	neutrophils	Est. 2.5 fold increase over control	PAK-PBD pulldown	Lewis et al., 2002. Am J Physiol Cell. Physiol. 282, C271
Interleukin-3	5 ug/ml for 5 min	MC/9 cells (mouse mast cell-like cell line)	2.4 fold increase over control. maximal activa-tion after 5 min and maintained for at least 10 min	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183
Interleukin-3	5 ug/ml for 5 min	primary bone marrow-derived mast cells (BMMCs)	4.3 fold increase over control	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183
colony stimulating factor-1 (CSF-1)	200 ng/ml, 1-10 min	WEHI 274.3, myelomonocytic cell line	maximal at 1 min (12 fold), signif drop by 5 min (8.5 fold) and at 10 min (4.5 fold) compared to control	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183
Steel locus factor (SLF)	50 ng/ml, 1-10 min	MC/9 cells	maximal at 1 min (7 fold increase over control) and dropped by 5-10 min (4 fold increase)	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183
Steel locus factor (SLF)	50 ng/ml, 5 min	primary bone marrow-derived mast cells (BMMCs)	3.5 fold increase over control	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183
granulocyte-macrophage colony-stimu-lating factor (GM-CSF)	10 ug/ml for 5 min	MC/9 cells (mouse mast cell-like cell line)	maximal at 1 min, maintained for at least 10 min	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183

Activators of Rho, Rac and Cdc42

Examples of known Cdc42 activators

Activator*	Treatment	Cell Line	Response	Type of Assay	Citation
Epidermal Growth Factor (Cat. # CN02)	100 ng/ml	Swiss 3T3 cells	Maximal activation after 1 min	Cdc42 G-LISA (Cat. # BK127)	In-house QC results
heregulin beta 1 (ligand for the ErbB3/ErbB4 tyrosine kinase-coupled receptors)	10 ng/ml	breast cancer cell lines MCF-7 and T-47D serum-starved for 48 h	maximal by 5 min. treated 1-60 min	PAK-PBD pulldown	Yang et al., 2006. Molec and Cellular Biol. 26, 831
hyperosmotic exposure	100 mM NaCl added into the isotonic medium for 10 min	neutrophils	7.2 fold increase over control	PAK-PBD pulldown	Lewis et al., 2002. Am J Physiol Cell Physiol. 282, C271
hyperosmotic exposure	200 mM sucrose added, a non-ionic osmolyte, for 10 min	neutrophils	Est. 7.2 fold increase over control	PAK-PBD pulldown	Lewis et al., 2002. Am J Physiol Cell Physiol. 282, C271
Interleukin-3	5 ug/ml for 5 min	primary bone marrow-derived mast cells (BMMCs)	2 fold increase over control	PAK-PBD pulldown	Grill and Schrader, 2002. Blood. 100, 3183