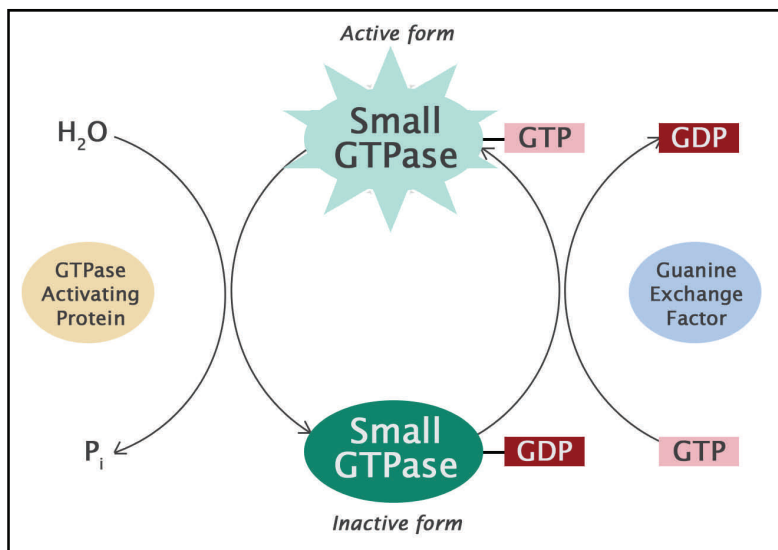


Small GTPase & G-Protein Signaling

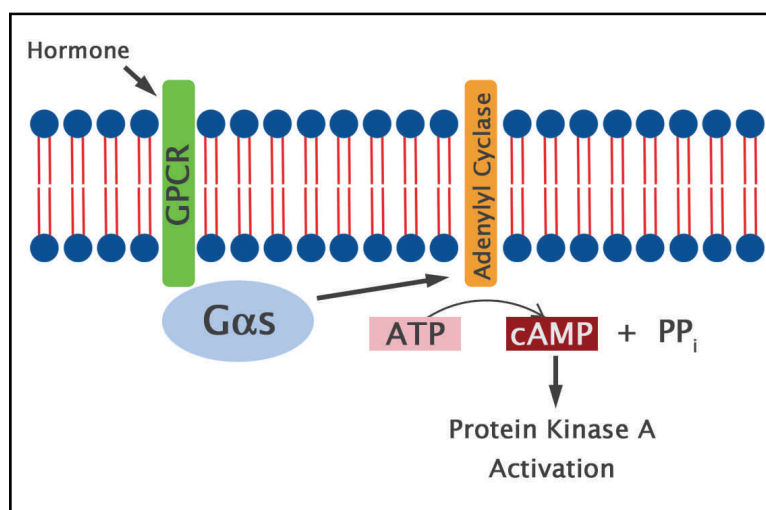


Small GTPase Assay Kits

Small GTPase Assay Beads

Rho Kinase Assays

Cyclic AMP/GMP Assays



Measure the active form of small GTPase proteins safely and easily

Small GTPase Activation Assays

Small GTP-binding proteins (GTPases) regulate a variety of cell signaling pathways and are therefore involved in a wide range of cell functions, processes and morphology. These proteins are found *in vivo* in both active (GTP) and inactive (GDP) forms. Guanine nucleotide exchange factors (GEFs) activate small GTPases by catalyzing them to release GDP and bind instead to GTP. The active form of each small GTPase binds selectively to a binding domain to regulate various signaling cascades.

Our Small GTPase Activation Assays use visible agarose beads containing a specific binding domain to selectively pull down the active (GTP) form of the target of interest. The precipitated GTPase is then detected by Western blot using a target-specific antibody included in the kit. These Small GTPase Activation Assay kits are safe and non-radioactive.

The visible Small GTPase Assay Beads are also available to order separately.



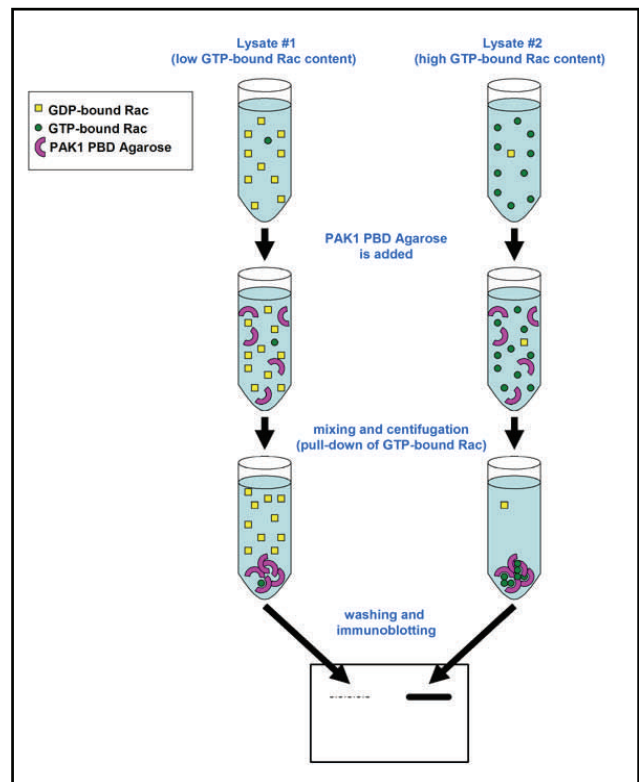
Visible Small GTPase Assay Beads.

Assays are available for the following targets:

- Arf1
- Arf6
- Rac1
- Rac2
- Ral
- Ran
- Rap1
- Rap2
- Cdc42
- H-Ras
- K-Ras
- N-Ras
- Pan-Ras
- RhoA
- RhoB
- RhoC

If you are studying more than one target, consider one of our Small GTPase combo kits:

- Rac1/Cdc42
- RhoA/Rac1/Cdc42

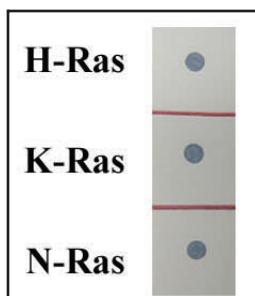


Small GTPase Activation Assay Principle.

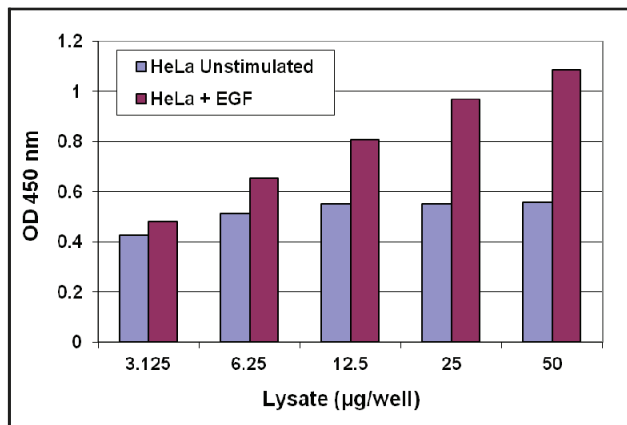
Quantify active Ras with higher throughput

96-Well Ras Activation ELISA Kits

Our 96-Well Ras Activation Assays use the Raf1 Rho binding domain (Raf1 RBD) to selectively pull down the active form of Ras from purified or endogenous samples. The captured GTP-Ras is then detected by a pan-Ras antibody and HRP-conjugated secondary antibody. Detection is by either colorimetric or chemiluminescent plate reader.



Pan-Ras Antibody Specificity. Anti-pan-Ras antibody reactivity with H-Ras, K-Ras and N-Ras human isoforms by dot blot.

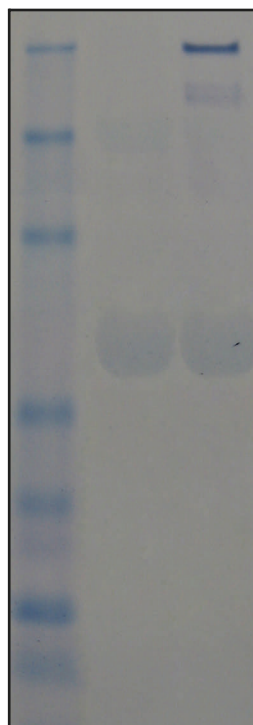


EGF Stimulation and Active Ras Detection with the 96-Well Ras Activation ELISA Kit. HeLa cells were serum starved for 18 hours before EGF stimulation of 50 ng/mL for 2 minutes. Lysates were then prepared according to the assay protocol.

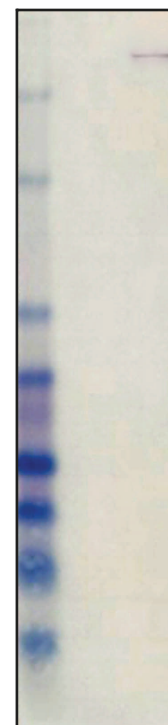
Active Rac-GEF Assay Kit (Tiam1)

Guanine nucleotide exchange factors (GEFs) activate small GTPases by catalyzing the exchange of GDP for GTP.

Our Active Rac-GEF Assay Kit (Tiam1) uses the agarose bead technology of our Small GTPase Activation Assays (previous page). Agarose beads pull down the active form of Rac-GEFs from endogenous lysates or purified samples. The specific GEF known as Tiam1 is then specifically detected with a polyclonal antibody.



Tiam1 Activation Assay. **Left:** 293 cells were transfected with active Tiam1. Active Tiam1 in lysate was pulled down with Rac1 G15A agarose beads. **Right:** Active Tiam1 in 2 mg of MDA-231 cell lysate was pulled down with Rac1 G15A agarose beads and probed with anti-Tiam1 antibody.

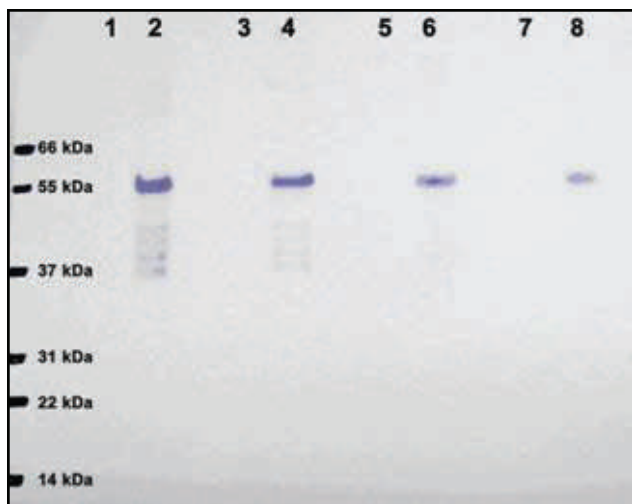


Quantify ROCK activity easily by Western blot or ELISA plate reader

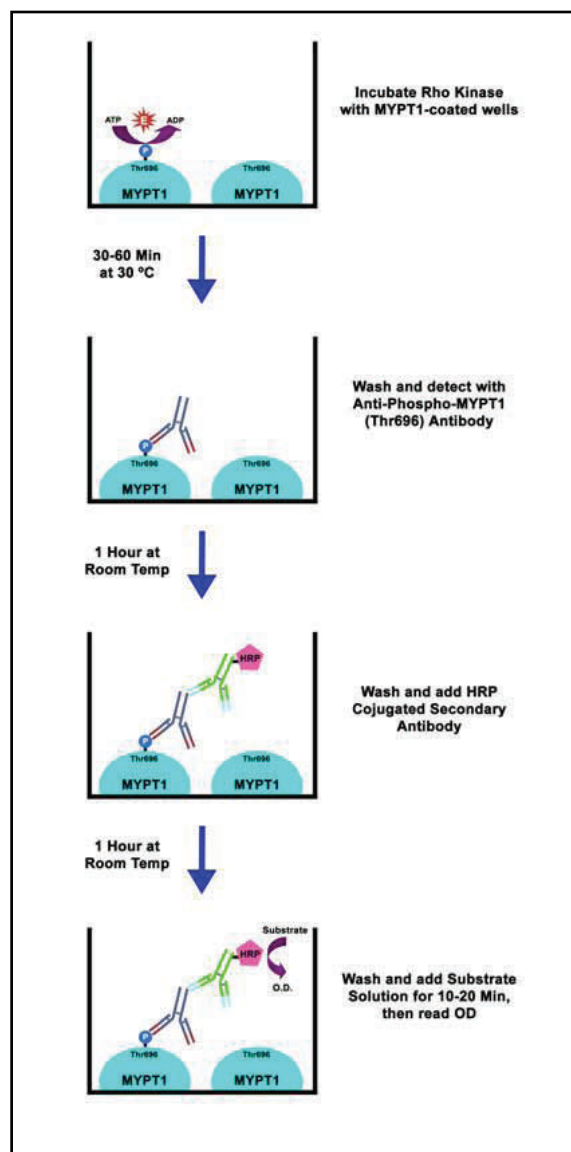
Rho Kinase (ROCK) Activity Assays

Rho, a small GTPase family member, is an essential regulator of signaling pathways. Its actions are mediated by various downstream effectors including Rho-associated Kinase (ROCK). ROCK mediates Rho signaling and reorganizes actin cytoskeleton through phosphorylation of various substrates. For example, ROCK inactivates myosin phosphatase through phosphorylation of the myosin phosphatase target subunit 1 (MYPT1).

Our ROCK Activity Assays use a non-radioactive format to measure the level of active ROCK in cell or tissue lysates by measuring the phosphorylation of MYPT1. The Immunoblot kit provides a convenient format for measuring ROCK activity in a few samples, while the 96-well activity assay kit contains a strip-well plate precoated with recombinant MYPT1 for higher throughput.



Results Using the ROCK Activity Immunoblot Kit. Lanes 1, 3, 5, 7: Without ROCK (negative control); Lanes 2, 4, 6, 8: With ROCK. Lanes 1 & 2: 200 ng MYPT1; Lanes 3 & 4: 100 ng; Lanes 5 & 6: 50 ng; Lanes 7 & 8: 25 ng.



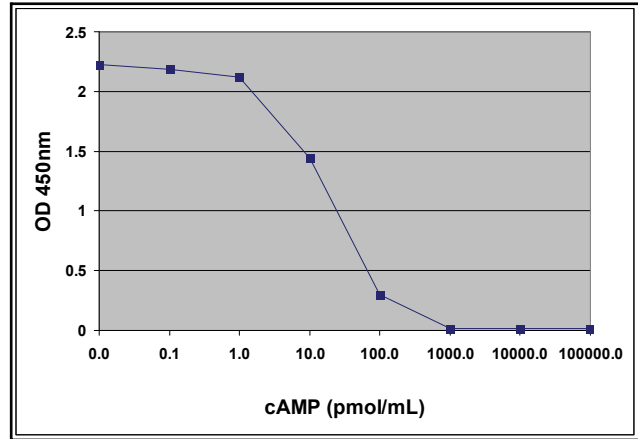
96-Well ROCK Activity Assay Principle.

Measure cAMP or cGMP levels with high sensitivity in a 96-well plate format

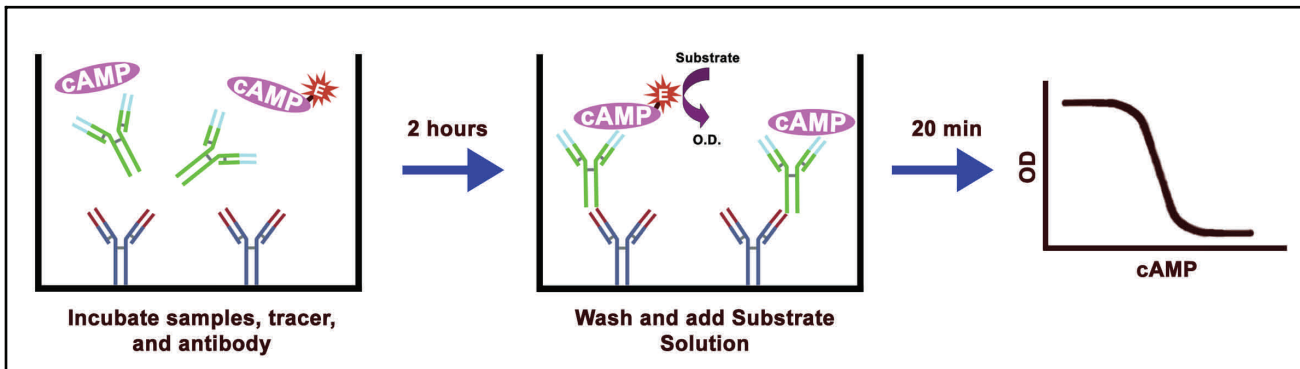
Cyclic AMP and GMP ELISA Kits

Cyclic AMP and cyclic GMP are important regulatory molecules in the G-protein signaling cascade. Our cAMP and cGMP ELISA Kits provide a highly sensitive method to measure low levels of cAMP or cGMP in a variety of sample types.

Each kit is available with two detection methods: colorimetric and chemiluminescent. Detect as little as 1 pmol/mL, even with the colorimetric format. The kits are suitable for use with cell and tissue lysates, urine, plasma, or culture medium. A convenient 8 x 12 strip-well plate format allows you to save unused wells of the plate for use at a later date.



Purification of Recombinant Ad-β-Gal Using ViraBind™ Adenovirus Purification Kit (#VPK-100).



Assay Principle for the cAMP ELISA Kit (Colorimetric Format).

Ordering Information and Published Citations

Small GTPase Activation Assays

Product Name	Detection	Size / Qty	Catalog Number
Arf1 Activation Assay	Immunoblot/ECL	20 Assays	STA-407-1
Arf6 Activation Assay	Immunoblot/ECL	20 Assays	STA-407-6
Cdc42 Activation Assay	Immunoblot/ECL	20 Assays	STA-402
Rac1 Activation Assay	Immunoblot/ECL	20 Assays	STA-401-1
Rac2 Activation Assay	Immunoblot/ECL	20 Assays	STA-401-2
Ral Activation Assay	Immunoblot/ECL	20 Assays	STA-408
Ran Activation Assay	Immunoblot/ECL	20 Assays	STA-409
Rap1 Activation Assay	Immunoblot/ECL	20 Assays	STA-406-1
Rap2 Activation Assay	Immunoblot/ECL	20 Assays	STA-406-2
H-Ras Activation Assay	Immunoblot/ECL	20 Assays	STA-400-H
K-Ras Activation Assay	Immunoblot/ECL	20 Assays	STA-400-K
N-Ras Activation Assay	Immunoblot/ECL	20 Assays	STA-400-N
Pan-Ras Activation Assay	Immunoblot/ECL	20 Assays	STA-400
RhoA Activation Assay	Immunoblot/ECL	20 Assays	STA-403-A
RhoB Activation Assay	Immunoblot/ECL	20 Assays	STA-403-B
RhoC Activation Assay	Immunoblot/ECL	20 Assays	STA-403-C

Recent Product Citations

- Ohashi, K. et al. (2012). Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS or MEK1. *PNAS* **109**:E2127-E2133. (STA-400)
- Camalier, C.E. et al. (2010). Elevated phosphate activates N-ras and promotes cell transformation and skin tumorigenesis. *Cancer Prev. Res.* **3**:359-370. (STA-400)
- Geryk-Hall, M. et al. (2010). Driven to death: inhibition of farnesylation increases Ras activity in osteosarcoma and promotes growth arrest and cell death. *Mol. Cancer Ther.* **9**:1111-1119. (STA-400)
- Harmon, B. et al. (2008). Induction of the Gαq signaling cascade by the human immunodeficiency virus envelope is required for virus entry. *J. Virol.* **82**:9191-9205. (STA-400)
- Yanagashita, T. et al. (2014). Actin-binding protein, espin: a novel metastatic regulator for melanoma. *Mol. Cancer Res.* **12**:440-446. (STA-401-1, STA-403-A)
- Tsukamoto, Y. et al. (2013). A novel heart failure mice model of hypertensive heart disease by angiotensin II infusion, nephrectomy, and salt loading. *Am. J. Physiol. Heart Circ. Physiol.* **305**:H1658-H1667. (STA-401-1)
- Petzold, T. et al. (2013). β1 integrin-mediated signals are required for platelet granule secretion and hemostasis in mouse. *Blood* **122**:2723-2731. (STA-401-1)
- Cristante, E. et al. (2013). Identification of an essential endogenous regulator of blood-brain barrier integrity, and its pathological and therapeutic implications. *PNAS* **110**:832-841. (STA-401-1)
- Richier, L. et al. (2010). NOS1AP associates with scribble and regulates dendritic spine development. *J. Neurosci.* **30**:4796-4805. (STA-401-1)
- Takano, A. et al. (2009). Identification of nectin-4 oncoprotein as a diagnostic and therapeutic target for lung cancer. *Cancer Res.* **69**:6694-9703. (STA-401-1)
- Shen, E. et al. (2009). Rac1 is required for cardiomyocyte apoptosis during hyperglycemia. *Diabetes* **58**:2386-2395. (STA-401-1)
- Lise, M-F. et al. (2009). Myosin-Va-interacting protein, RILPL2, controls cell shape and neuronal morphogenesis via Rac signaling. *J. Cell Sci.* **122**:3810-3821. (STA-401-1)
- Zhan, L. et al. (2008). Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. *Cell* **135**(5):865-878. (STA-401-1)
- Biname, F. et al. (2008). Transforming growth factor β controls the directional migration of hepatocyte cohorts by modulating their adhesion to fibronectin. *Mol. Biol. Cell* **19**:945-956. (STA-401-1)
- He, S. et al. (2013). SRGAP1 is a candidate gene for papillary thyroid carcinoma susceptibility. *J. Clin. Endocrinol. Metab.* **98**:E973-E980. (STA-402)
- Zhang, S. et al. (2008). The tumor suppressor LKB1 regulates lung cancer cell polarity by mediating Cdc42 recruitment and activity. *Cancer Res.* **68**:740-748. (STA-402)
- Fiuza, M. et al. (2013). GluN3A expression restricts spine maturation via inhibition of GIT1/Rac1 signaling. *PNAS* **110**:20807-20812. (STA-403-A)
- Basu, M. et al. (2012). Wnt/β-catenin pathway is regulated by PITX2 homeodomain protein and thus contributes to the proliferation of human ovarian adenocarcinoma cell SKOV-3. *J. Biol. Chem.* **288**:4355-4367. (STA-403-A)
- Hayes, N.V.L. et al. (2010). Expression or neuregulin 4 splice variants in normal human tissues and prostate cancer and their effects on cell motility. *Endocr. Relat. Cancer* **18**:39-49. (STA-403-A)
- Godin, C.M. et al. (2010). The angiotensin II type 1 receptor induces membrane blebbing by coupling to RhoA, Rho kinase, and myosin light chain kinase. *Mol. Pharmacol.* **77**:903-911. (STA-403-A)
- Grossman, A. et al. (2013). The small GTPase ARF6 stimulates β-catenin transcriptional activity during WNT5A-mediated melanoma invasion and metastasis. *Sci Signal* **6**:ra14. (STA-407-6)

Ordering Information and Published Citations

Small GTPase Activation Assay Combo Kits

Product Name	Detection	Size / Qty	Catalog Number
RhoA/Rac1/Cdc42 Activation Assay Combo Kit	Immunoblot/ECL	3 x 10 Assays	STA-405
Rac1/Cdc42 Activation Assay Combo Kit	Immunoblot/ECL	2 x 20 Assays	STA-404

Recent Product Citations

- Holmes, K.M. et al. (2012). Insulin-like growth factor-binding protein 2-driven glioma progression is prevented by blocking a clinically significant integrin, integrin-linked kinase, and NF- κ B network. *PNAS* **109**:2168-2173. (STA-404)
- Chen, H. et al. (2010). Integrity of SOS1/EPS8/ABI1 tri-complex determines ovarian cancer metastasis. *Cancer Res.* **70**:9979-9990. (STA-404)
- Sultana, H. et al. (2010). Anaplasma phagocytophilum induces actin phosphorylation to selectively regulate gene transcription in Ixodes scapularis ticks. *J. Exp. Med.* **10.1084/jem.20100276**. (STA-404)
- Wang, J. et al. (2013). DEK depletion negatively regulates Rho/ROCK/MLC pathway in non-small cell lung cancer. *J. Histochem. & Cytochem.* **10.1369/00221155413488120**. (STA-405)
- Quint, P. et al. (2013). Sphingosine 1-phosphate (S1P) receptors 1 and 2 coordinately induce mesenchymal cell migration through S1P activation of complementary kinase pathways. *J. Biol. Chem.* **288**:5398-5406. (STA-405)
- Baranwai, S. et al. (2011). Molecular characterization of the tumor-suppressive function of nischarin in breast cancer. *J. Natl. Cancer Inst.* **10.1093/jnci/djr350**. (STA-405)
- Tian, D. et al. (2010). Antagonistic regulation of actin dynamics and cell motility by TRPC5 and TRPC6 channels. *Sci. Signal.* **3**:ra77. (STA-405)
- Xu, Y. et al. (2010). Neuropilin-2 mediates VEGF-C-induced lymphatic sprouting together with VEGFR3. *J. Cell Biol.* **118**:115-130. (STA-405)
- Ma, T. et al. (2010). Viral G protein-coupled receptor up-regulates angiopoietin-like 4 promoting angiogenesis and vascular permeability in Kaposi's sarcoma. *PNAS* **104**:14363-14368. (STA-405)

96-Well Ras Activation ELISA Kits

Product Name	Detection	Size / Qty	Catalog Number
96-Well Ras Activation ELISA Kit	Colorimetric	96 Assays	STA-440
	Chemiluminescent	96 Assays	STA-441

Small GTPase and GEF Assay Beads

Product Name	Target	Size / Qty	Catalog Number
GGA3 PBD Agarose Beads	Arf	400 μ g	STA-419
PAK1 PBD Agarose Beads	Cdc42, Rac	400 μ g	STA-411
Raf1 RBD Agarose Beads	Ras	400 μ g	STA-410
RalBP1 PBD Agarose Beads	Ral	400 μ g	STA-420
RalGDS RBD Agarose Beads	Rap	400 μ g	STA-418
RanBP1 Agarose Beads	Ran	400 μ g	STA-421
Rhotekin RBD Agarose Beads	Rho	400 μ g	STA-412
Cdc42 G15A Agarose Beads	Cdc42-GEF	800 μ g	STA-433
Rac1 G15A Agarose Beads	Rac1-GEF	800 μ g	STA-432
RhoA G17A Agarose Beads	RhoA-GEF	400 μ g	STA-431

Recent Product Citations

- Yuen, H. et al. (2013). RanGTPase: a candidate for Myc-mediated cancer progression. *J. Natl. Cancer Inst.* **105**:475-488. (STA-410)
- Moniz, S. et al. (2007). Protein kinase WNK2 inhibits cell proliferation by negatively modulating the activation of MEK1/ERK1/2. *Oncogene* **26**(41):6071-6081. (STA-410)
- Pothula, S. et al. (2013). Regulation of Cdc42 expression and signaling is critical for promoting corneal epithelial wound healing. *Invest. Ophthalmol. Vis. Sci.* **54**:5343-5352. (STA-411)
- Sabbatini, M.E. et al. (2010). CCK activates RhoA and Rac1 differentially through G-alpha-13 and G-alpha-q in mouse pancreatic acini. *Am. J. Physiol. Cell Physiol.* **298**:C592-C605. (STA-411)
- Zhang, Q.-G. et al. (2009). Estrogen attenuates ischemic oxidative damage via an estrogen receptor alpha-mediated inhibition of NADPH oxidase activation. *J. Neurosci.* **29**:13823-13836. (STA-411)
- Levy-Adam, F. et al. (2008). Heparanase facilitates cell adhesion and spreading by clustering of cell surface heparan sulfate proteoglycans. *PLoS One* **3**(6):e2319. (STA-411)
- Sabbatini, M. et al. (2008). Rap1 activation plays a regulatory role in pancreatic amylase secretion. *J. Biol. Chem.* **283**:23884-23894. (STA-411, STA-412)
- Colacios, C. et al. (2011). The p.Arg63Trp polymorphism controls Vav1 functions and Fox3p regulatory T cell development. *J. Exp. Med.* **208**:2183-2191. (STA-432)

Ordering Information and Published Citations

Active Rac-GEF Assay Kit (Tiam1)

Product Name	Detection	Size / Qty	Catalog Number
Active Rac-GEF Assay Kit (Tiam1)	Immunoblot/ECL	20 Assays	STA-422

Recent Product Citation

Oubaha, M. et al. (2012). Formation of a PKC/ β -catenin complex in endothelial cells promotes angiotensin-1-induced collective directional migration and angiogenic sprouting. *Blood* **120**:3371-3381.

Rho Kinase (ROCK) Activity Assays

Product Name	Detection	Size / Qty	Catalog Number
ROCK Activity Immunoblot Kit	Immunoblot	20 Assays	STA-415
96-Well ROCK Activity Assay	Colorimetric	96 Assays	STA-416

Recent Product Citations

- Georgess, D. et al. (2014). Comparative transcriptomics reveals RhoE as a novel regulator of actin dynamics in bone-resorbing osteoclasts. *Mol. Biol.* **25**:380-396. (STA-415)
- Wang, J.N. et al. (2011). Response gene to complement 32 promotes vascular lesion formation through stimulation of smooth muscle cell proliferation and migration. *Arterioscler. Thromb. Vasc. Biol.* **31**:e19-e26. (STA-415)
- Li, Z. et al. (2009). TrkB1 induces liver metastasis of pancreatic cancer cells by sequestering Rho GDP dissociation inhibitor and promoting RhoA activation. *Cancer Res.* **69**:7851-7859. (STA-415)
- Xiao, L. et al. (2009). ROCK mediates phorbol ester-induced apoptosis in prostate cancer cells via p21-Cip1 upregulation and JNK. *J. Biol. Chem.* **284**:29365-29375. (STA-415)
- Yotova, I.Y. et al. (2011). Abnormal activation of Ras/Raf/MAPK and RhoA/ROCKII signaling pathways in eutopic endometrial stromal cells of patients with endometriosis. *Hum. Reprod.* **10.1093/humrep/der010**. (STA-416)
- Burger, D. et al. (2011). Endothelial microparticle formation by angiotensin II is mediated via ang II receptor type I/NADPH oxidase/Rho kinase pathways targeted to lipid rafts. *Arterioscler. Thromb. Vasc. Biol.* **31**:1898-1907. (STA-416)
- Haas, B. et al. (2009). Protein kinase G controls brown fat cell differentiation and mitochondrial biogenesis. *Sci. Signal.* **2**:ra78. (STA-416)

Cyclic AMP and GMP Assays

Product Name	Detection	Size / Qty	Catalog Number
cAMP ELISA Kit	Colorimetric	96 Assays	STA-500
		5 x 96 Assays	STA-500-5
	Chemiluminescent	96 Assays	STA-501
		5 x 96 Assays	STA-501-5
cGMP ELISA Kit	Colorimetric	96 Assays	STA-505
		5 x 96 Assays	STA-505-5
	Chemiluminescent	96 Assays	STA-506
		5 x 96 Assays	STA-506-5

Recent Product Citations

- Meyer, R. et al. (2013). GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *PNAS* **110**:9529-9534. (STA-500)
- Sun, Z. et al. (2011). The WD40 repeat protein WDR26 binds G β g and promotes G β g-dependent signal transduction and leukocyte migration. *J. Biol. Chem.* **286**:43902-43912. (STA-500)
- Chen, M. et al. (2010). Involvement of cAMP in nerve growth factor-triggered p35/Cdk5 activation and differentiation in PC12 cells. *Am. J. Physiol. Cell Physiol.* **299**:C516-C527. (STA-500)
- McCoy, K.L. et al. (2010). PAR1 and PAR2 couple to overlapping and distinct sets of G proteins and linked signaling pathways to differentially regulate cell physiology. *Mol. Pharmacol.* **77**:1005-1015. (STA-500)
- Liu, L. et al. (2012). Radil controls neutrophil adhesion and motility through β 2-integrin activation. *Mol. Biol. Cell* **23**:4751-4765. (STA-501)