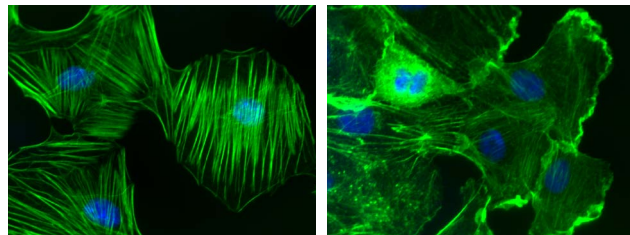




## About Activation Assays

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, and Ral. Also, the quantifiable GLISA versions enabled a new wave of more sensitive applications, e.g. measurement in limited primary cell numbers and Matrigel 3D matrix. We continue to develop and maintain these high standards, which allow you to produce the best results in the least amount of time.



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).

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 GLISA® 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.



**Activation Assay Video**

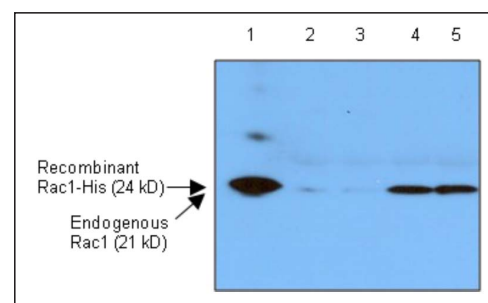
Learn which assay format is right for you.  
[www.cytoskeleton.com/activationassayvideo](http://www.cytoskeleton.com/activationassayvideo)

## Comparison of Pull-down and GLISA 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

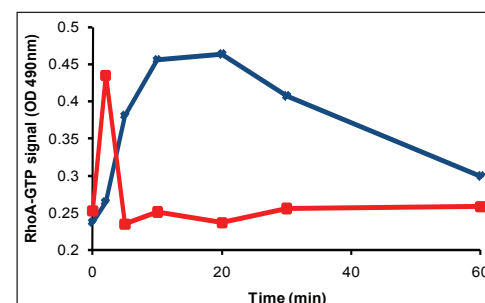
\* Numerical readouts and fewer sample handling steps make G-LISA® assays more quantitative.

## 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 µg of lysate were assayed with 10 µg 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® 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)**  
Chen W. et al., 2016. *Oncol. Lett.* **11**, 1375-1381.  
Jones E.L. et al., 2016. *J. Immunol.* DOI: 10.4049/jimmunol.1500357.

**Rac1,2,3 G-LISA® (Cat. # BK125)**  
Qin G. et al., 2016. *Cancer Lett.* **374**, 85-95.  
Marusak C. et al., 2016. *Pharmacol. Res.* **113**, 515-520.

**Rac1 G-LISA® (Cat. # BK128)**  
Ubba V. et al., 2016. *Reprod. Sci.* DOI: 10.1177/1933719116669057.  
Sayyad W.A. et al., 2016. *PLoS One.* **11**, e0146842.

**Cdc42 G-LISA® (Cat. # BK127)**  
Jones E.L. et al., 2016. *J. Immunol.* DOI: 10.4049/jimmunol.1500357.  
Croise P. et al., 2016. *Endocr. Relat. Cancer.* **23**, 281-293.

**Ras G-LISA® (Cat. # BK131)**  
L.D. Camargo et al., 2013. *Free Radic. Biol. Med.* **65**, 1398-1407.  
S.-J. Lee et al., 2013. *J. Biol. Chem.* **288**, 25244-25253.

**More online!**

[www.cytoskeleton.com/activation-assays](http://www.cytoskeleton.com/activation-assays)

## 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 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 BK034	20 assays 50 assays
Rac1 Activation Assay Biochem Kit™	BK035-S BK035	20 assays 50 assays
RalA Activation Assay Biochem Kit™	BK040	50 assays
Ras Activation Assay Biochem Kit™	BK008-S BK008	20 assays 50 assays
RhoA Activation Assay Biochem Kit™	BK036-S BK036	20 assays 80 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.

ELISA	Cat. #	Amount
Total RhoA ELISA	BK150	96 assays

See Pg. 13

## G-LISA® 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 Activation Assay Bundle BK135=BK124-S+BK127-S+BK128-S	BK135	3 Kits (24 assays/kit)
Arf1 G-LISA® Activation Assay, colorimetric	BK132	96 assays
Arf6 G-LISA® Activation Assay, colorimetric	BK133	96 assays
Cdc42 G-LISA® Activation Assay, colorimetric	BK127-S BK127	24 assays 96 assays
Rac1,2,3 G-LISA® Activation Assay, colorimetric	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	BK128-S BK128	24 assays 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 BK124	24 assays 96 assays
RhoA G-LISA® Activation Assay, luminescence	BK121	96 assays
Protease Inhibitor Cocktail (100x stock)	PIC02	1 ml

### Activators & Inhibitors

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

See Pg. 10

### Acti-stain Phalloidins

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

See Pg. 13

### GTPase Affinity Beads & Proteins

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

See Pg. 10