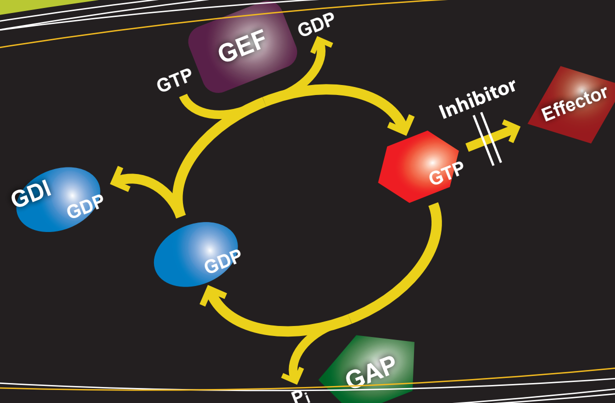
Helping advance science, one protein at a time.

CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

AUG 2011



this issue

Measure Active Small G-proteins

G-LISA® Publication Spotlight

RhoA G-LISA® Activation Assay

G-LISA® Products

Upcoming Meetings

Neuroscience	Nov 12-16
AACR-NCI-EORTC	Nov 12-16
ASCB	Dec 3-7

Cytoskeleton Products

- Actin Proteins
- Antibodies
- Activation Assays
- ELISA Kits
- G-LISA® Kits
- Pull-down Assays
- Motor Proteins
- Small-G-Proteins
- Tubulin Proteins

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Accurate measurement of activated Small G-proteins

Measuring the activation (i.e. the level of GTP-loading) of small GTPases in cells or tissues has become a widely used technique for signal transduction research in recent years across multiple disciplines.

Traditionally, this has been done by pull-down methods which are time-consuming and require large amounts of starting material. This laborious technique is limited in the number of samples that can be handled simultaneously and yields only semi-quantitative results. With the introduction of the G-LISA® activation assays, Cytoskeleton Inc., has revolutionized the activation assay field with kits that accurately measure GTPase activation with only a fraction of the starting material that pull-downs require in less than 3 hours!

But measuring activated levels of a small G-protein only tells half the story. Naturally, the next question, asked by both reviewers and yourself, is … *is my treatment altering the total expression level of the small G-proteins?* When measuring total GTPase levels, scientists have either used Western blotting with its well-known shortcomings or qPCR. The latter technique is particularly flawed in that qPCR does not directly measure protein levels, only mRNA levels. Recently a paper by Boulter et al. from Keith Burridge's laboratory (Nature Cell Biol, 2010, v 12, p 477) clearly demonstrated the hazards of employing qPCR to measure mRNA levels and making inferences about the level of translated protein. Boulter et al. found that changes in Rho protein levels occur independently of Rho's mRNA levels.

To fill this technique gap, Cytoskeleton Inc., created the Total RhoA ELISA (Cat. # BK150) as the perfect complement to the RhoA G-LISA® activation assay (Cat. # BK124). These two assays use the same lysate to provide the researcher with the fastest and most sensitive method to fully quantify RhoA protein, both its active and inactive forms, without the time-consuming hassles of Western blotting and inaccuracies of qPCR.

Publication Spotlight

Cytoskeleton's G-LISA® products are utilized across the diverse scientific fields of cancer, neuroscience and cell biology. Check out these recent papers!

Kogashiwa et al., 2010. Docetaxel suppresses invasiveness of head and neck cancer cells in vitro. *Cancer Science.* v 101, p 1382.

Sumida and Stamer, 2010. Sphingosine-1 phosphate enhancement of cortical actomyosin organization in cultured human Schlemm's canal endothelial cell monolayers. *Invest Ophthalmol Vis Sci.* v 51, p 6633.

Zhao et al., 2010. Lovastatin inhibits EGFR dimerization and Akt activation in squamous cell carcinoma cells: potential regulation by targeting rho proteins. *Oncogene.* v 29, p 4682.

Godsel et al., 2010. Plakophilin2 couples actomyosin remodeling to desmosomal plaque assembly via RhoA. *Molec Biol Cell.* v 21, p 2844.

Deramaudt et al., 2011. FAK phosphorylation at Tyr-925 regulates cross-talk between focal adhesion turnover and cell protrusion. *Molec Biol Cell.* v 22, p 964.

Helping advance science, one protein at a time.

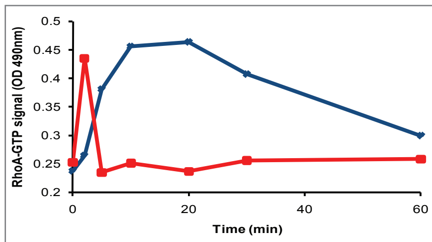
G-LISA® PRODUCTS

Accurate Simple protocol Flexible Format

Visit Cytoskeleton.com for more information on our expansive G-LISA® product line.

RhoA G-LISA®

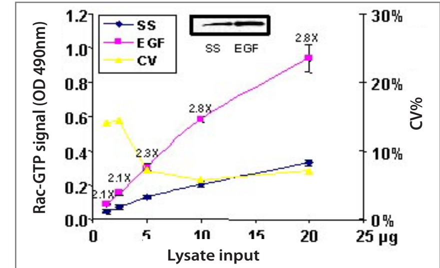
Product	Cat #	Amount
RhoA G-LISA® Activation Assay, colorimetric	BK124	96 assays
RhoA G-LISA® Activation Assay, luminescence	BK121	96 assays



Time course of activation of RhoA in Swiss 3T3 cells by CN01 and LPA. Serum-starved Swiss 3T3 cells were treated with Rho Activator, cat. # CN01 (blue diamonds) or LPA (magenta squares). RhoA activity was measured by reading signals at OD_{490nm}.

Rac1 and Rac1,2,3 G-LISA®

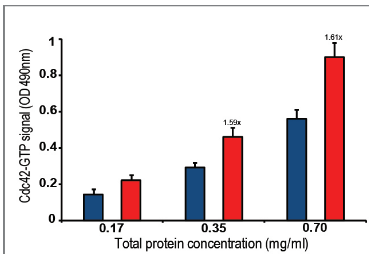
Product	Cat #	Amount
Rac1,2,3 G-LISA® Activation Assay, colorimetric	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	BK128	96 assays



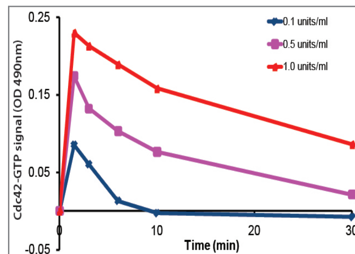
Rac activation by EGF measured by the G-LISA® kit, cat. # BK125. Serum-starved Swiss 3T3 cells were incubated with Rac Activator cat. # CN02 (0.25 units/ml for 2 min). 20, 10, 5, 2.5 µg of lysates were subjected to the G-LISA® assay and read at OD_{490nm}. 500 µg of the same lysates were subjected to the traditional PAK pull-down assay (shown in inset).

Cdc42 G-LISA®

Product	Cat #	Amount
Cdc42 G-LISA® Activation Assay, colorimetric	BK127	96 assays



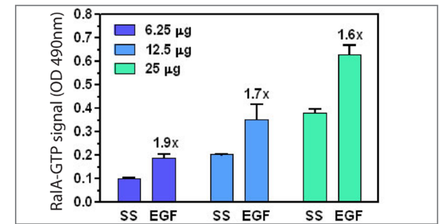
Swiss 3T3 cell extracts assayed for Cdc42 activity with BK127. Cells were serum-starved (SS) for 16 h with 1% serum and 8 h with 0% serum and treated with 0.25 unit/ml CN02 (Cdc42 Activator) for 2 min. 12.5, 25 and 75 µg of lysates were used per well. Red bars = activated, blue = serum- starved. Numbers on top of yellow bars represent fold activation.



Time course of Cdc42 activation with CN02. Swiss 3T3 cells were serum-starved (SS) for 16 h with 1% serum and 8 h with 0% serum and treated with CN02 (0.1, 0.5 and 1.0 units/ml for 1.5, 3.0, 6.0, 10 and 30 min). Cell lysates were subjected to the Cdc42 G-LISA™ assay and read at OD_{490nm}. The "controlled state" serum-starved value (0.22) was subtracted from these samples prior to plotting. At 1.0 unit/ml the total activation was 2.05 fold or 105% over the controlled state at 1.5 min.

RalA G-LISA®

Product	Cat #	Amount
RalA G-LISA® Activation Assay, colorimetric	BK129	96 assays

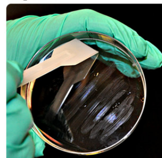


RalA activation by EGF measured by G-LISA®. Rat-2 cells were serum-starved (SS) for 24 h and treated with EGF (100 ng/ml for 2 min). Lysates from these cells were tested at 25 µg/well, 12.5 µg/well and 6.25 µg/well starting concentrations in the Ral G-LISA® assay. Absorbance was measured at OD_{490nm}.

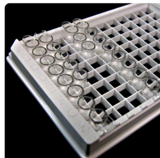
Simple and Quick Protocol

Choose Number of Wells

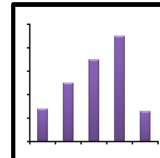
Prepare Your Lysate



Perform Assays



Collect Your Data



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