

vosin Proteins

# Cytoskeleton, Inc. Minicatalog 2018

Actin Proteins Simplicity P S Signal-Seeker" Actin Proteins WB Processor **Motor Proteins Tubulin Proteins** Live Cell Imaging Small G-proteins **Custom Services Fubulin Proteins** Signal-Seeker<sup>™</sup> Kits Activation Assays GOBlot<sup>™</sup> WB Processor Spirochrome<sup>™</sup> Bioprobes Custom Services G-LISA<sup>™</sup> Activation Assays **Pull-down Activation Assays** 

### Helping advance science one protein at a time.

## **New Products Inside!**

GEF Proteins and Assays Signal-Seeker<sup>™</sup> Kits and Antibodies Spirochrome<sup>™</sup> Live Cell Probes

Antibodies

Proteins

# Table of Contents

#### New Products - 3

Exciting new products to help you discover more about posttranslation modifications and measure GTP exchange on K-Ras.

#### GOBlot<sup>™</sup> WB Processor - 6



NEW

The first affordable Western blot processor. Save hours a day with this fully automated device.

### Activation Assays - 8,9

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

#### Antibodies - 12

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

#### Actin Biochem Kits<sup>™</sup> - 14

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

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

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

#### Motor Werks<sup>™</sup> - 18

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



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

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



### Live Cell Imaging Reagents - 7

Actin, ECM, DNA, lysosome and tubulin bio-probes, and small G-protein activators and inhibitors.

#### Small G-protein Tools - 10, 11

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

#### Actin Visualization - 13

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



### Actin & ECM Proteins - 15

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

### Tubulin & FtsZ Proteins - 17

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

### Custom Services - 19

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





## **Online Resources at Cytoskeleton.com**

- Promotions and Discounts
- Technical Resources
- Informational Newsletters
- Video Guides
- Protocols
- Webinars





For worldwide customers, contact your distributor at cytoskeleton.com/distributors











# **New Products**

## Signal-Seeker<sup>™</sup> Kits

The First Comprehensive PTM Detection Kits For Non-PTM Experts



- Comprehensive kits affinity & control beads, de-PTM inhibitors, validation antibody, lysis & wash buffers, etc.
- Measure endogenous signaling events.
- Includes Cytoskeleton's new Acetyl-Lysine Detection Kit, that was built with the novel technology found in our popular Ub, SUMO 2/3 and pY kits.

See p. 4-5 for more information

## **New GEF and K-Ras Proteins**

Measuring inhibition of SOSI GEF induced GDP dissociation from K-Ras4B wild type and mutants.



Legend: Inhibitors of GTP Exchange Factors (GEFs), which target small G-proteins such as Ras and Rho, are identified in a kinetic assay with either On or Off rates of GTP or GDP, or by measuring the equilibrium of the nucleotides to indicate a preference for GDP (inactive) or GTP (active).

- Reliable, pure, biologically active GEF proteins and small G-proteins.
- SOS1, Ras-GRF, Tiam1, Vav1, Vav2, and ARNO available.
- K-Ras4B mutant proteins; G12V, G12D, G13D, G13S, Q61P, K128A, R135A, plus compound binding site mutants G12D+D38A and G12D +I36N.
- Custom compound screening with K-Ras wild type and mutated isoforms with or without exchange factors.

See p. 10,11 & 19 for more information

### Signal-Seeker<sup>™</sup> Antibodies New Acetyl-Lysine Antibodies



- Validated for immunofluorescence applications.
- High affinity & specificity for Acetyl-lysine PTMs.

Check out our full line of Signal-Seeker antibodies for Ac, Phosphorylation, Ubiquitin, and SUMO 2/3 PTMs.

#### See p. 12 for more information

## **Community Spotlight**



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 2018!

cytoskeleton.com

## 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 available UBD that effectively enriches mono- and poly-ubiquitinated proteins.
- First to develop an Acetyl-Lysine antibody that visualizes acetylated mitochondria by immunofluorescence.

## A New Level of Excellence

#### Superior Acetylated Protein Enrichment



Densitometry Measurement (TSA treated)	73250	28846	18379	24383
Number of unique bands (TSA treated)	++++	++	+	++

Figure Legend: **(A)** Various acetyl-lysine affinity reagents were used to IP acetylated proteins from Cos-7 cells either treated (+) or untreated (-) with TSA (1 $\mu$ M) and nicotinamide (1mM) for 6 hours. IPs were performed according to the manufacturer's instructions. Western blot was performed using AAC03-HRP antibody (1:3000). **(B)** Top row: quantitation of Western immunoblot by densitometry. Bottom row: visual analysis of unique bands per TSA-treated condition.

rtoskeleton.com

#### 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

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, endogenous changes, because it is critical to determine if these PTM events are occuring physiologically.



Acetylated proteins (green): AAC02 (1:500), anti-mouse 488 Actin (Red): Rhodamine Phalloidin Nucleus (Blue): DAPI

Application	Product	Validation Data
Western Blot	Acetyl-Lysine Antibody-HRP labeled, Cat. # AAC03-HRP Acetyl-Lysine Mouse Antibody (7B5A1), Cat. # AAC02 Acetyl-Lysine Mouse Antibody (19C4B2.1), Cat. # AAC03 Acetyl-Lysine Mouse Antibody (3C6.08.20), Cat. # AAC01	Yes Yes Yes Yes
Immunofluoresence	Acetyl-Lysine Mouse Antibody (785A1). Cat. # AAC02 Acetyl-Lysine Mouse Antibody (19C4B2.1), Cat. # AAC03 Acetyl-Lysine Mouse Antibody (3C6.08.20), Cat. # AAC01	Yes Yes Yes
Immunoprecipitation	Signal-Seeker <sup>™</sup> Acetyl-Lysine Detection Kit, Cat. # BK163 Signal-Seeker <sup>™</sup> Acetyl-Lysine Detection Kit, Cat. # BK163-S Acetyl-Lysine Affinity Beads, Cat. # AAC04-beads Acetyl-Lysine Mouse Antibody (7B5A1), Cat. # AAC02 Acetyl-Lysine Mouse Antibody (19C4B2.1), Cat. # AAC03 Acetyl-Lysine Mouse Antibody (3C6.08.20), Cat. # AAC01	Yes Yes Yes Yes Yes Yes
Chromatin IP	Acetyl-Lysine Mouse Antibody (785A1), Cat. # AAC02 Acetyl-Lysine Mouse Antibody (19C4B2.1), Cat. # AAC03 Acetyl-Lysine Mouse Antibody (3C6.08.20), Cat. # AAC01	Yes Yes Yes

\*Recommended products for each application are highlighted in blue





#### Signal-Seeker<sup>™</sup> Ubiquitin Tools

#### **Highlights:**

- · First ubiquitin enrichment tool (Cat. # UBA01-beads) that isolates both endogenous monoand poly-ubiquitination without heavy and light chain contamination.
- Ubiquitination detection kit (Cat. # BK161) contains affinity beads, control beads, inhibitors, and the complete lysis, dilution, and wash buffer system to maximize Ub detection.
- HRP-labeled ubiquitin antibody (Cat. # AUB01-HRP) simplifies Ub detection.

#### Identification of mono- and poly-ubiquitinated PD-L1

#### References

1. Horita H. et al. 2017. Identifying regulatory post-translational modifications of PD-L1: a focus on mono-ubiquitination. Neoplasia. DOI: 10.1016/i.neo.2017.02.0062 2. Braganza A. et al. 2017. UBE3B Is a Calmodulin-regulated, Mitochondrionassociated E3 Ubiquitin Ligase. J Biol Chem. Doi: 10.1074/ibc.M116.766824. 3. Rahman H.N.A. et al. Selective Targeting of a Novel Epsin-VEGFR2 Interaction Promotes VEGF-Mediated Angiogenesis. Cir Res. Doi: 10.1161/CIRCRESAHA.115.307679.



Legend: The Signal-Seeker Ubiquitination Detection Kit (Cat. # BK161) was used to examine endogenous PD-L1 ubiquitination in response to EGF stimulation. A431 cells were harvested with BlastR lysis buffer with or without NEM. Lysates were incubated with ubiquitin affinity beads and analyzed for ubiquitinated PD-L1. The image shows a Western blot result using anti-PD-L1 antibody. Note: Both mono- and polyubiquitinated forms of PD-L1 were detected. Adapted from Ref 1.

#### Signal-Seeker<sup>™</sup> SUMOylation 2/3 Tools

#### **Highlights:**

- SUMOylation 2/3 detection kits (Cat. # BK162) overcome the most challenging pitfalls<sup>2</sup> encountered when studying SUMO 2/3 modified proteins (Please see our white paper).
- Highly validated SUMO 2/3 affinity beads (Cat. # ASM24-beads) isolate a rich profile of SUMO2/3 proteins for identification with downstream applications.
- Our SUMO 2/3 antibody (Cat. # ASM23) was developed in-house, and was shown to be more robust than other commerical SUMO 2/3 antibodies.

#### References

1. Horita H. et al. 2017. A simple toolset to identify endogenous post-translational modifications for a target protein: a snapshot of the EGFR signaling pathway. Bioscience Reports. 37 BSR20170919 DOI: 10.1042/ BSR20170919.

2. Barysch S. et al. 2014. Identification and analysis of endogenous SUMO1 and SUMO2/3 targets in mammalian cells and tissues using monoclonal antibodies. Nat Protoc, 9(4):896-909.



#### Optimizied detection of SUMO 2/3 modified proteins

Legend: The Signal-Seeker SUMO 2/3 Detection Kit (Cat. BK162) was used to examine endogenous SUMO 2/3. A431 cells were lysed with BlastR lysis buffer (Cytoskeleton Inc.), RIPA lysis buffer, or 1% SDS denaturing lysis buffer all with deSUMOylase inhibitors (NEM+TPEN). Lysate was incubated with SUMO 2/3 affinity beads or IgG control beads. Samples were analyzed for TFII-I, RANGAP1, or Ubc9 SUMOylation by Western.

#### Signal-Seeker<sup>™</sup> Phosphotyrosine Tools

#### **Highlights:**

- Easily study target protein tyrosine phosphorylation with the comprehensive Phospho-Tyrosine Detection Kit (Cat. # BK160). No mass spectrometry required.
- Broad spectrum phosphotyrosine affinity beads (Cat. # APY03-beads), which can be used for tyrosine phosphorylation studies by mass spectrometry.
- Unique phosphotyrosine antibody (Cat. # APY03) detects a broad range of tyrosinephosphorylated proteins.

#### References

1. Yusuf M.Z. et al. 2017. Prostacyclin reverses platelet stress fibre formation causing platelet aggregate instability. Sci Rep. Doi: 10.1038/s41598-017-05817-9.

2. Kaukonen R. et al. 2016. Normal stroma suppresses cancer cell proliferation via mechanosensitive regulation of JMJD1amediated transcription. Nat Commun. Doi: 10.1038/ncomms12237.

3. Horita H. et al. 2017. A simple toolset to identify endogenous post-translational modifications for a target protein:a snapshot of the EGFR signaling pathway. Bioscience Reports, 37 BSR20170919.

Measurement of rapidly changing levels of Rac1 tyrosyl phosphorylation.



Legend: Anti-Rac1 antibody (Cat. # ARC03) was used to probe a blot containing samples from a time course of A431 cells treated with EGF. Time is noted in minutes at head of lane, IN = total Rac1 in extract. All other lanes indicate the concentrated immuno-precipitation purified phospho-form of Rac1 isolated by the Signal -Seeker Kit.

## Signal-Seeker<sup>™</sup> Toolkit Products

#### NEW Acetyl-Lysine Products

Description	Amount	ltem #
Signal-Seeker <sup>™</sup> 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	ltem #
Signal-Seeker <sup>™</sup> Ubiquitination Detection Kit	30 assays	BK161
Signal-Seeker <sup>™</sup> 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
NEW Ubiquitin Mouse Antibody-HRP labeled	1 x 100 μl	AUB01-HRP

#### SUMOylation 2/3 Products

Description	Amount	ltem #
Signal-Seeker <sup>™</sup> SUMO 2/3 Detection Kit	30 assays	BK162
Signal-Seeker <sup>™</sup> 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
NEW SUMO 2/3 Mouse Antibody-HRP labeled	1 x 100 μl	ASM23-HRP

#### Phosphotyrosine Products

Description	Amount	ltem #
Signal-Seeker <sup>™</sup> Phosphotyrosine Detection Kit	30 assays	BK160
Signal-Seeker <sup>™</sup> 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 Mouse Antibody-HRP labeled	1 x 100 μl	APY03-HRP

#### BlastR<sup>™</sup> Lysis System

Description	Amount	Item #
BlastR™ Rapid Lysate Filter System	50 assays	BLR01
BlastR™ Rapid Lysate Filters	50 assays	BLR02

# GOBlot<sup>™</sup> Western Blot Processor

# **<u>G</u>OBlot**

## The First Affordable Western Blot Processor

## Save Time and Money

- Save up to 3 hours per day
- Fully automated
- Choice of four routines
- Choice of six colors
- Recycle primary antibody
- No obligation trial period



## Development of the GOBlot

The GOBlot<sup>™</sup> Western Blot Processor (patents pending) was developed to be an affordable and helpful device for all scientists. With input from over 300 research scientists, the flexible routines and capabilities of the processor were defined. Western blot automation with the GOBlot saves the average researcher 3 hours a day while improving the reproducibility of results. The GOBlot saves researchers money by providing the option to recycle primary antibody and it does not require machine-specific consumables. See the results for yourself with reviews online at Biocompare.com.

GOBlot Options	Cat. #
GOBlot (1 Machine)	WBM01
GOBlot (4 Machine Bundle)	WBM01

## Recycle the Primary Antibody

Original 1st recycle 2nd recycle 3rd recycle



Legend: A single anti-RhoA monoclonal antibody solution was used to probe four replicate membranes. Note how the 3rd re-cycle test has a reduced band intensity indicating the antibody is being depleted by the repeated process.

## See the GOBlot in Action



Use the QR code or visit www.cytoskeleton.com/gobl<mark>ot</mark>

Order online today and start saving time tomorrow! Ask about the no obligation trial period by e-mailing tservice@cytoskeleton.com



# Live Cell Imaging Reagents



Top Left: STED image of dorsal root ganglion (rat) stained with SiR-actin. Courtesy of Elisa D'Este, MPI Biophysical Chemistry, Göttingen. Top Right: fMCF10A cells expressing H2B-GFP (Blue) in Matrigel stained with SiR-actin (red). Image taken on an inverted LSM microscope. Courtesy of Christian Conrad and Katharina Jechow, Heidelberg. Lower Left: HeLa cells expressing H2B-mCherry (Blue) stained with SiR-Tubulin (Red), courtesy of Daniel Gerlich and Claudia Blaukopf, Inst. Mol. Biotech., Vienna). Lower Right: STED image of cultured rat hippocampal neurons stained with SiR-actin. Actin rings (stripes) with 180 nm periodicity can be seen. Courtesy Of Elisa D'Este, MPI Biophysical Chemistry, Göttingen.



www.cytoskeleton.com/spirochrome

#### **Tubulin Imaging**

Description	Ex / Em	Cat. #	Amount
SiR700-Tubulin Kit Includes SiR700-Tubulin and Verapamil	690 / 720 nm	CY-SC014	35 nmol
SiR-Tubulin Kit Includes SiR-Tubulin and Verapamil	630 / 680 nm	CY-SC002	50 nmol
<b>Cytoskeleton Kit</b> Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	CY-SC006	50 nmol each
AMCA Labeled Tubulin	350 / 440 nm	TL440M-A TL440M-B	5 x 20 μg 20 x 20 μg
HiLyte Fluor™ 488 Labeled Tubulin	460 / 520 nm	TL488M-A TL488M-B	5 x 20 μg 20 x 20 μg
TRITC Rhodamine Labeled Tubulin	535 / 590 nm	TL590M-A TL590M-B	5 x 20 μg 20 x 20 μg
X-Rhodamine Labeled Tubulin	560 / 620 nm	TL620M-A TL620M-B	5 x 20 μg 20 x 20 μg
HiLyte Fluor™ 647 Labeled Tubulin	620 / 670 nm	TL670M-A TL670M-B	5 x 20 μg 20 x 20 μg

## 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
Fibronectin	na	FNR03-A	5 x 20 μg
Biotinylated		FNR03-B	20 x 20 μg
Laminin	535 / 590 nm	LMN01-A	5 x 20 μg
Red fluorescent, rhodamine		LMN01-B	20 x 20 μg
Laminin	460 / 520 nm	LMN02-A	5 x 20 μg
Green fluorescent, HiLyte Fluor <sup>™</sup> 488		LMN02-B	20 x 20 μg
Laminin	na	LMN03-A	5 x 20 μg
Biotinylated		LMN03-B	20 x 20 μg

SiR-Actin, Sir-Tubulin, SiR-DNA, SiR-Lysosome are trademarks of Spirochrome SA (Switzerland). HiLyteFluor is a trademark of Anaspec Inc. (CA, USA).



Top Left: Swiss 3T3 cells treated with cell permeable Rho inihibitor (Cat. # CT04) and stained with rhodamine phalloidin (Cat. PHDR1); note the lack of F-actin stress fibers in each cell. Top right: Fluorescent fibronectin (Cat. # FNR01) treated MCF10A cells (image kindly provided by A. Varadara and M. Karthykenyan, Univ. S. Carolina, Columbia, SC). Lower left: 488 HiLyte Fluor™ labeled tubulin polymerized *in vitro* (Cat. # TL488M). Lower right: Fluorescent non-muscle actin (Cat.# APHR) injected in to CHO cells; note the stress fibers across the whole cell width (kindly provided by Dr. Goldman's lab, NorthWestern Univ, Chicago, IL).

#### Small G-protein Modulators and Actin Imaging

Description	Ex / Em	Cat. #	Amount
SiR700-Actin Kit Includes SiR-Actin and Verapamil	690 / 720 nm	CY-SC013	35 nmol
SiR-Actin Kit Includes SiR-Actin and Verapamil	630 / 680 nm	CY-SC001	50 nmol
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	CY-SC006	50 nmol each
Rhodamine Actin Protein	535 / 590 nm	APHR-A	4 x 10 μg
Human platelet, non-muscle		APHR-C	20 x 10 μg
<b>Rhodamine Actin protein</b>	535 / 590 nm	AR05-B	10 x 20 μg
Rabbit skeletal muscle		AR05-C	20 x 20 μg
Rho Activator II		CN03-A	3 x 20 μg
Deamidation of Rho Gln-63		CN03-B	9 x 20 μg
<b>Rho Inhibitor I</b> ADP ribosylation of Rho Asn-41		CT04-A CT04-B CT04-C	1 x 20 μg 5 x 20 μg 20 x 20 μg
Rho/Rac/Cdc42 Activator I		CN04-A	3 x 20 μg
Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61		CN04-B	9 x 20 μg
<b>Rho Activator I</b>		CN01-A	5 x 10 units
SHP-2 phosphatase-mediated Rho activation		CN01-B	20 x 10 units
Rac/Cdc42 Activator II		CN02-A	5 x 10 units
EGF receptor-mediated Rac/Cdc42 activation		CN02-B	20 x 10 units

#### Lysosome Imaging

Lysosome Live Cell Imaging Reagents	Ex / Em	Cat. #	Amount
SiR-Lysosome Kit Includes SiR-Lysosome and Verapamil	630 / 680 nm	CY-SC012	50 nmol
SiR700-Lysosome Kit Includes SiR700-Lysosome and Verapamil	690 / 720 nm	CY-SC016	35 nmol

#### **DNA** Imaging

DNA Live Cell Imaging Reagents	Ex / Em	Cat. #	Amount
SiR-DNA Kit Includes SiR-DNA and Verapamil	630 / 680 nm	CY-SC007	50 nmol
SiR700-DNA Kit Includes SiR700-DNA and Verapamil	690 / 720 nm	CY-SC015	35 nmol



## Activation Assays

#### 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.





Learn which assay format is right for you. www.cytoskeleton.com/activationassayvideo

#### **Example Product Citations**

#### RhoA G-LISA® (Cat. # BK124)

Chen G.P. et al. 2017. *Mol. Med. Rep.* 15, 3153-3160. Dai D.P. et al. 2017. *Cell Biol. Int.* doi: 10.1002/ cbin.10795.

#### Rac1 G-LISA® (Cat. # BK126)

Kai M. et al. 2017. *Mol. Carcinog.* 56, 1743-1752. Jones M.M. et al. 2017. *Mol. Oral Microbiol.* 32, 375-389.

#### Rac1 G-LISA® (Cat. # BK128)

Kumar V. et al. 2017. *Sci Rep.* 7, 1885. Peretti A.S. et al. 2017. *Am. J. Pathol.* doi: 10.1016/j. ajpath.2017.10.018.

#### Cdc42 G-LISA® (Cat. # BK127)

Hernandez A.J.A. et al. 2018. *Toxicology*. 394, 35-44. Rafiq N.B. et al. 2017. *J. Cell Biol*. 216, 181-197.

#### Ras G-LISA® (Cat. # BK131)

Huang J.L. et al. 2017. *Nat. Commun.* 8, 15144. Arora P. et al. 2017. *Hepatology.* doi: 10.1002/ hep.29049.

#### More online!

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.



### 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

#### **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

### < Acti-stain Phalloidins

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

See Pg. 13

### < 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. 10

## 😪 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



# 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 Mechanism	Protein Modulation	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 Specific inhibitor of Rho activity, ADP ribosylation of Rho Asn-41 (very cell permeable)	Cell permeable	Direct	CT04-A CT04-B CT04-C	1 x 20 μg 5 x 20 μg 20 x 20 μg
<b>C3 Transferase Protein</b> Specific inhibitor of Rho activity, ADP ribosylation of Rho Asn-41 (limited cell permeability)	Pinocytosis	Direct	СТ03-А СТ03-С	1 x 25 μg 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
NEW ARNO Protein Sec7 GEF domain protein. GEF for Arf1 & 6. Human recomb., 6xHis tag	>85%	CS-GE07 CS-GE07-XL	3 x 20 μg 1 x 200 μg
Dbs His Protein, RhoGEF domain (DH/PH) GEF for Cdc42 and RhoA	>80%	GE01-A	2 x 50 µg
<b>p50RhoGAP GST Protein, full length</b> GAP for Cdc42, Rac, and Rho	>90%	GAP01-A GAP01-B	1 x 50 μg 4 x 50 μg
<b>p50RhoGAP GST Protein, GAP domain</b> GAP for Cdc42, Rac, and Rho	>90%	GAS01-A GAS01-B	1 x 50 μg 4 x 50 μg
NEW Ras-GRF GEF protein Cdc25 domain Human recomb., MBP tagged	>85%	CS-GE03	1 x 100 µg
RhoGDI GST Protein Inhibitor of Cdc42, Rac, and Rho	>90%	GDI01-A	1 x 25 µg
SOS1 Ras GEF Domain Protein GEF for H-, K- or N-Ras	>90%	CS-GE02 CS-GE02-XL	1 x 100 μg 1 x 1 mg
NEW Tiam1 GEF protein, GEF for Rac. Human recomb. DHPH domain, MBP tag	>85%	CS-GE04	1 x 100 µg
NEW Vav1 GEF protein, GEF for Rac. Human recomb. DHPHC1 domain Y174D mutant, 6xHis tagged	>85%	CS-GE05	1 x 100 µg
NEW Vav2 GEF protein , GEF for Rac. Human recomb. DH domain, 6xHis tagged	>85%	CS-GE06	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
GGA3-PBD Beads Binds active (GTP-bound) Arf1 and Arf6	>85%	GGA05-A	1 x 500 µg
PAK-PBD Protein	>80%	PAK01-A	1 x 250 μg
Binds active (GTP-bound) Cdc42 and Rac1,2,3		PAK01-B	4 x 250 μg
PAK-PBD Beads	>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 Binds active (GTP-bound) RhoA,B,C	>90%	RT01-A	1 x 500 µg
Rhotekin-RBD Beads	>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.

10 cytoskeleton.com

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 µl 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.



RhoA ELISA Kit results. The plot indicates the linear dependence of the OD<sub>490nm</sub> absorbance with the concentration of RhoA.

Product	Cat. #	Amount
<b>Total RhoA ELISA</b> Measures total RhoA levels	BK150	96 assays

### 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 Antibodies	Host	Туре	Species Reactivity	Cat.#	Amount
Cdc42 Specific Antibody Human Cdc42 Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ACD03 ACD03-S	2 x 200 μl 1 x 50 μl
Rac1 Specific Antibody Human C-terminal Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARC03-A ARC03-S	2 x 100 μl 1 x 50 μl
RhoA Specific Antibody Human RhoA Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARH04 ARH04-S	2 x 100 μl 1 x 50 μl

### Additional Signal Transduction Reagents

Signal Transduction Reagents	Cat.#	Amount
RhoGAP Assay Biochem Kit™	BK105	80-160 assays
<b>GTΡγ5</b> Non-hydrolyzable GTP analog, 50 μl of 20 mM	BS01	1 x 500 µg
GTPase CytoPhos™ Assay One step assay for enzyme Kcat 0.01 to 100	BK054	1000 assays

**Bulk Discounts Available** 

Visit www.cytoskeleton.com/bulk

### RhoGEF Assay Biochem Kit<sup>™</sup>

Fluorophore-based assay measures fluorescent-GTP nucleotide exchange on small G-proteins.

- Mant-GTP included, Bodipy-GTP<sup>™</sup> can be substituted.
- Reaction buffer included
- · Cdc42, Rac1, and RhoA GTPase control proteins
- GEF domain of Dbs (Positive control for GTP or GDP exchange on RhoA and Cdc42)
- Useful with all other small G-proteins



RhoGEF assay using BK100. Cdc42 alone and Cdc42 + Dbs was incubated and fluorescence intensity (nucleotide exchange) was measured.

avs

roduct	Cat.#	Amou
hoGEF Exchange Assay Biochem Kit™	BK100	60-300 ass

### **Purified G-proteins**

R

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 CD01-C CD01-XL	1 x 100 μg 3 x 100 μg 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 RC01-C RC01-XL	1 x 100 μg 3 x 100 μg 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 RS01-C	1 x 100 μg 3 x 100 μg
NEW K-Ras4B Protein, human rec., wild-type	>90%	CS-RS03	1 x 100 µg
NEW K-Ras4B Protein, human rec., G12V mutant	>90%	CS-RS04	1 x 100 µg
NEW K-Ras4B Protein, human rec., other mutant	>90%	various	various
NEW N-Ras Protein, human rec., wild type	>90%	CS-RS02	1 x 100 µg
NEW 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 RH01-C RH01-XL	1 x 100 μg 3 x 100 μg 1 x 1 mg
RhoC His Protein, wild-type	>90%	RH03-A	1 x 100 µg

# 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

rtoskeleton.com

Small G-protein Antibodies	Host	Туре	Species Reactivity	Cat. #	Amount
<b>Cdc42 Specific Antibody</b> Human Cdc42 Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ACD03 ACD03-S	2 x 200 μl 1 x 50 μl
Rac1 Specific Antibody Human C-terminal Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARC03 ARC03-S	2 x 100 μl 1 x 50 μl
<b>RhoA Specific Antibody</b> Human RhoA Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARH04 ARH04-S	2 x 100 μl 1 x 50 μl

#### 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



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 AAC01-S	2 x 100 μl 1 x 25 μl
NEW Acetyl Lysine Affinity Beads	Mouse	mAB	IP	AAC04-beads	5 x 500 µl
Phosphotyrosine Antibody	Mouse	mAb	WB, IP, IF, ELISA	APY03 APY03-S	2 x 100 μl 1 x 25 μl
Anti-Phosphotyrosine Affinity Beads	Mouse	mAb	IP	APY03-Beads	4 x 300 µl
Phosphotyrosine Antibody (HRP conjugate)	Mouse	mAb	WB	APY03-HRP APY03-HRP-S	1 x 100 μl 1 x 25 μl
NEW SUMO-2/3 Antibody (Clone 12F3)	Mouse	mAb	WB, IF, IP	ASM23 ASM23-S	2 x 100 μl 1 x 25 μl
NEW SUMO-2/3 Antibody (Clone 11G2)	Mouse	mAb	IF, IP	ASM24 ASM24-S	2 x 200 μl 1 x 150 μl
NEW SUMO-2/3 Affinity Beads	Mouse	mAb	IP	ASM24-Beads	2 x 400 µl
Ubiquitin Antibody	Mouse	mAb	WB, IF	AUB01 AUB01-S AUB01-XL	2 x 100 μl 1 x 25 μl 4 x 500 μl
Ubiquitin Affinity Beads (binds mono-/poly-ubiquitin tagged proteins)	n/a	n/a	IP	UBA01-beads	2 x 120 μl
NEW Control for Ippt IgG Beads	n/a	n/a	IP	CIG01-beads	10 assays
NEW Control for Ubiquitin	n/a	n/a	IP	CUB01	10 assays

### Cytoskeleton Protein Antibodies

Antibodies	Host	Туре	Applications	Cat. #	Amount
Actin Antibody	Rabbit	pAb	WB, IP	AAN01-A AAN01-B	1 x 100 µg 3 x 100 µg
Tubulin Polyclonal Antibody	Sheep	pAb	WB, IF, ELISA	ATN02 ATN02-S	2 x 100 μl 1 x 25 μl
Cofilin Antibody	Rabbit	pAb	WB, IP, ICC	ACFL02-A ACFL02-B	1 x 50 μg 3 x 50 μg
Profilin Antibody	Rabbit	pAb	WB, ICC, ELISA, IP	APUF01-A	1 x 50 μg

## **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™ Fluorescent Phalloidins

Product	Excitation	Emission	Signal stability without antifade* (Ti/2 in secs)	Cat. #	Amount**
Acti-stain <sup>™</sup> 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 <sup>™</sup> 555 phalloidin	535 nm	585 nm	46	PHDH1-A	300 Slides
Acti-stain™ 670 phalloidin	640 nm	670 nm	18	PHDN1-A	300 Slides
SiR700-Actin Kit Includes SiR-Actin and Verapamil	690 nm	720 nm	na***	CY-SC013	35 nmol
SiR-Actin Kit Includes SiR700-Actin and Verapamil	630 nm	680 nm	na***	CY-SC001	50 nmol

\* 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. \*\*\* 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™

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<sup>™</sup>.

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

#### Live Cell Actin Staining Products

In living cells, actin structures can be observed by incorporating fluorescently labeled actin, by expressing GFP-actin, or by expressing a fluorescently labeled actin binding protein sub-domain. Fluorescent actin is the most accurate reporter of actin structures.



Rhodamine-labeled actin microinjected into CHO cells. The labeled actin (Cat. # APHR) rapidly incorporates into the cellular actin cytoskeleton and allows real time observation of actin dynamics.

Labeled Actins	Source	Purity	Cat. #	Amount
Rhodamine Actin Protein	Human platelet, non-muscle	>99%	APHR-A APHR-C	4 x 10 μg 20 x 10 μg
Rhodamine Actin Protein	Rabbit skeletal muscle	>99%	AR05-B AR05-C	10 x 20 μg 20 x 20 μg
Spirochrome™ SiR-Actin Kit	Chemical	>99%	CY-SC001	50 nmol
Spirochrome™ SiR700-Actin Kit	Chemical	>99%	CY-SC013	35 nmol

SiR-Actin is a trademarks of Spirochrome SA (Switzerland).

# 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> Assay Biochem Kit™	BK037	30-100 assays
Protease Inhibitor Cocktail (100x solution)	PIC02	1 ml

### 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- Down Assay Biochem Kit <sup>™</sup> (skeletal muscle actin)	BK001	30-100 assays
Actin Binding Protein Spin-Down Assay Biochem Kit" (non-muscle actin)	BK013	30-100 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 Assay Biochem Kit™	BK003	30-100 assays

### F-actin Visualization Biochem Kit™

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 also provided in the kit.

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



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<sup>™</sup>.



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

### Labeled Actin Proteins

Labeled Actins	Source	Purity	Cat. #	Amount
Biotinylated Actin Protein	Rabbit skeletal muscle	>99%	AB07-A AB07-C	5 x 20 μg 20 x 20 μg
Pyrene Actin Protein	Rabbit skeletal muscle	>99%	AP05-A AP05-B	1 x 1 mg 5 x 1 mg
Rhodamine Actin Protein	Human platelet, non- muscle	>99%	APHR-A APHR-C	4 x 10 μg 20 x 10 μg
Rhodamine Actin Protein	Rabbit skeletal muscle	>99%	AR05-B AR05-C	10 x 20 μg 20 x 20 μg

### Actin Antibodies

Antibodies	Antigen	Host	Grade	Cat. #	Amount
Actin Antibody	C-terminal of actin	Rabbit	Affinity Purified	AAN01-A AAN01-B	1 x 100 µg 3 x 100 µg
Cofilin Antibody	N-terminal of human cofilin1	Rabbit	Affinity Purified	ACFL02-A ACFL02-B	1 x 50 μg 3 x 50 μg
Profilin Antibody	Purified human profilin	Rabbit	Affinity Purified	APUF01-A	1 x 50 µg

### **Bulk Discounts Available**

Visit www.cytoskeleton.com/bulk

### **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 AT01-C	2 x 50 μg 10 x 50 μg
Arp2/3 Protein Complex	Porcine brain	>90%	RP01P-A RP01P-B	2 x 50 μg 6 x 50 μg
Cofilin Protein	Recombinant human cofilin 1	95%	CF01-A CF01-C	1 x 100 μg 4 x 100 μg
Gelsolin Protein	Recombinant human, plasma isoform	>95%	HPG6-A HPG6-B	4 x 20 μg 20 x 20 μg
Myosin II Cardiac Protein	Bovine cardiac muscle	95%	MY03-A MY03-B	5 x 1 mg 20 x 1 mg
S1 Myosin Protein	Rabbit skeletal muscle Chymotrypsin digest of Cat. # MY02 plus chromat	>90%	CS-MYS04	1 x 250 μg
S1 Myosin Protein	Bovine cardiac muscle Chymotrypsin digest of Cat. # MY03 plus chromat.	>90%	CS-MYS03	1 x 250 μg
Heavy Meromyosin Protein	Bovine cardiac muscle Chymotrypsin digest of Cat. # MY03 plus FPLC.	90%	CS-MH03	1 x 100 µg
Myosin II Protein	Rabbit skeletal muscle	95%	MY02-A MY02-B	5 x 1 mg 20 x 1 mg
Heavy Meromyosin Protein	Rabbit skeletal muscle Chymotrypsin digest of Cat. # MY02.	90%	MH01-A	4 x 50 μg
Profilin Protein	Recombinant human profilin 1	>95%	PR02-A PR02-B PR02-XL2	1 x 100 μg 1 x 500 μg 1 x 1 mg
WASP protein VCA Domain: Acti- vates Arp2/3, GST tag.	Recombinant human	>95%	VCG03-A	1 x 500 μg

### Labeled ECM Proteins

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

#### Actin Buffers

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



### 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
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >99% pure tubulin	BK006P	24-30 assays
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >97% pure tubulin	BK004P	24-30 assays
Tubulin Polymerization Assay Biochem Kit™ Fluorescence-based, >99% pure tubulin	BK011P	96 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 T333P-B T333P-XL	5 x 20 μg 20 x 20 μg 1 x 500 μg
Microtubule Binding Protein Spin-Down Assay Biochem Kit™	BK029	30-100 assays

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

The Microtubule/Tubulin *In Vivo* Assay Kit measures the ratio of microtubules to tubulin in cell and tissue extracts. Samples are homogenized in lysis buffer, centrifuged, and then supernatant (tubulin) and pellet (microtubules) samples are run on a SDS-PAGE gel, blotted onto a membrane, and probed with anti-tubulin antibody. The tubulin antibody is ideal for dual and triple staining because the host animal is sheep, thus creating additional bandwidth for immunostaining.

Tubulin Biochem Kits™	Cat. #	Amount
Microtubule / Tubulin In Vivo Assay Biochem Kit™ Quantitates <i>in vivo</i> ratio of tubulin polymers & monomers	BK038	30-100 assays
Tubulin polyclonal antibody (host: sheep) Detects all species and isoforms of tubulin	ATN02 ATN02-S	2 x 100 μl 1 x 25 μl

### Specialized Tubulins For Pathogen Targeting

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
HeLa Cancer Cell Tubulin Protein (90% βΙ, 10% βΙV isotypes)	CS-H001-B	1 x 250 µg
HeLa Cancer Cell Tubulin Protein (biotinylated) (90% βl, 10% βlV isotypes)	H003	1 x 40 µg
MCF-7 Cell Tubulin Protein (55% βl, 6% βlII, 39% βlV isotypes)	CS-H005	1 x 250 µg



Tubulin & FtsZ Proteins



### **Pre-formed Microtubules**

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

#### MT+MAPs MT+BSA MT only Ρ S S P S Ρ kDa € MAP2 200 116 66 € BSA 55 Tubuli 36 31 -

**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).

### Unlabeled Tubulin Proteins

Unlabeled Proteins	Source	Purity	Cat. #	Amount
<b>Tubulin Protein</b> Lyophilized (no glycerol)	Porcine Brain	>99%	T240-A T240-B T240-C T240-DX	1 x 1 mg 5 x 1 mg 20 x 1 mg 1 x 10 mg
<b>Tubulin Protein, MAP rich</b> Lyophilized (no glycerol)	Porcine Brain	70% tubulin 30% MAPs	ML116-A ML116-B ML116-DX	1 x 1 mg 5 x 1 mg 1 x 10 mg
Tubulin for HTS Applications	Porcine Brain	97%	HTS03-A HTS03-B	1 x 4 mg 1 x 40 mg
<b>Tubulin Protein</b> Frozen (no glycerol)	Porcine Brain	>99%	T238P-A T238P-B T238P-C	1 x 1 mg 5 x 1 mg 20 x 1 mg
Microtubules pre-formed, lyophilized	Porcine brain	>99%	MT002-A MT002-XL	4 x 500 μg 1 x 10 mg
Caki-1 Tumor Tubulin Protein	Caki-1 Tu- mor Tissue	>90%	CS-TM001	1 x 250 µg
Cancer Cell Tubulin Protein	HeLa cells	>90%	CS-H001-B	1 x 250 µg
Cancer Cell Tubulin Protein	MCF-7 cells	>90%	CS-H005	1 x 250 µg

#### **Bulk Discounts Available**

inquire to tservice@cytoskeleton.com

### **FtsZ** Proteins

FtsZ Proteins	Source	Purity	Cat. #	Amount
FtsZ Protein	S. <i>aureus</i> , recombinant, 6xHis-tagged	>90%	FTZ02-A FTZ02-B	1 x 1 mg 5 x 1 mg
FtsZ Protein	S. pneumoniae, recombinant, 6xHis-tagged	>90%	FTZ03-A FTZ03-B	1 x 1 mg 5 x 1 mg
FtsZ Protein	<i>E. faecalis,</i> recombinant, 6xHis-tagged	>90%	FTZ04-A FTZ04-B	1 x 1 mg 5 x 1 mg
FtsZ Protein	<i>E. coli,</i> recombinant, 6xHis-tagged	>90%	FTZ05-A FTZ05-B	1 x 1 mg 5 x 1 mg

### Tubulin Buffers, Reagents, & MAPs

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

### Labeled Tubulin Proteins



HiLyte Fluor<sup>™</sup> 488 Labeled Tubulin - Cat. # TL488M HiLyte Fluor is a trademark of Anaspec, Inc. (CA).



TRITC Rhodamine Labeled Tubulin - Cat. # TL590M

La	abeled Tubulin Proteins	Ex / Em wavelength	T <sub>1/2</sub> of fluorescene (s)	Source	Purity	Cat. #	Amount
A	MCA Labeled Tubulin	350 +/-20 nm 440 +/-20 nm	10	Porcine Brain	>99%	TL440M-A TL440M-B	5 x 20 μg 20 x 20 μg
Hi La	iLyte Fluor™ 488 Ibeled Tubulin	460 +/-20 nm 520 +/-20 nm	300	Porcine Brain	>99%	TL488M-A TL488M-B	5 x 20 μg 20 x 20 μg
TR La	RITC Rhodamine Ibeled Tubulin	535 +/-20 nm 590 +/-20 nm	50	Porcine Brain	>99%	TL590M-A TL590M-B	5 x 20 μg 20 x 20 μg
X- La	Rhodamine Ibeled Tubulin	560 +/- 20 nm 620 +/- 20 nm	70	Bovine Brain	>99%	TL620M-A TL620M-B	5 x 20 μg 20 x 20 μg
Hi La	iLyte Fluor™ 647 Ibeled Tubulin	620 +/-20 nm 670 +/-20 nm	80	Porcine Brain	>99%	TL670M-A TL670M-B	5 x 20 μg 20 x 20 μg
Bi	otin Tubulin	na	na	Porcine Brain	>99%	T333P-A T333P-B T333P-XL	5 x 20 μg 20 x 20 μg 1 x 500 μg
Bi	otin Cancer Tubulin	na	na	HeLa cells	>90%	H003	1 x 40 µg

#### Kinesin & Dynein Proteins

Kinesin & Dynein Proteins	Source	Purity	Cat. #	Amount
CENP-E Motor Domain Protein	H. sapiens	>85%	CP01-A CP01-XL	2 x 25 μg 1 x 1 mg
Chromokinesin Motor Domain Protein	H. sapiens	>85%	CR01-A	2 x 25 µg
Dynein (cytoplasmic)	Porcine brain	>80%	CS-DN01	1 x 50 µg
Eg5 Motor Domain Protein	H. sapiens	>85%	EG01-A EG01-B EG01-XL	2 x 25 μg 10 x 25 μg 1 x 1 mg
Eg5 Homolog BimC Motor Domain Protein	A. nidulans	>85%	BM01-A	2 x 25 µg
Eg5 Homolog BimC Motor 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 Domain Protein	H. sapiens	>85%	KR01-A KR01-XL	2 x 25 μg 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 MP01-XL	2 x 25 μg 1 x 1 mg
MKLP2 Motor Domain Protein	H. sapiens	>85%	CS-MP05	1 x 50 µg

#### 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 MY02-B	5 x 1 mg 20 x 1 mg	
Myosin Cardiac Muscle Protein	Bovine	>95%	MY03-A MY03-B	5 x 1 mg 20 x 1 mg	
Heavy Meromyosin Skeletal Muscle Protein	Rabbit	80%	MH01-A	4 x 50 µg	
NEW Heavy Meromyosin Cardiac Muscle Protein	Bovine	80%	CS-MH03	1 x 100 µg	
Pre-formed F-actin filaments	Rabbit	>99%	AKF99-A AKF99-B	1 x 1 mg 5 x 1 mg	
NEW         Actin Thin Filaments (cardiac)           Calcium sensitive complex of F-actin tropomyosin α/β & Troponin C,I,T	Bovine	>90%	CS-TFC01	1 x 1 mg	
NEW         Actin Thin Filaments (skeletal)           Calcium sensitive complex of F-actin tropomyosin α/β & Troponin C,I,T	, Rabbit	>90%	CS-TFC02	1 x 1 mg	
<b>Tropomyosin / Troponin Complex</b> 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	
NEW Thin Filament Protein (tropomyosin/tra and actin, calcium activated myosin AT	opomodulir Pase	CS-	TFC01	1 x 1 mg	
Microtubules, Pre-formed, lyophilized, porcine source, substrate for kinesin ATPa	se assays	MTO	002-A 002-XL	4 x 500 μg 1 x 10 mg	
Actin Filaments, Pre-formed, lyophilized	assavs	AKF	99-A	1 x 1 mg	

 Paclitaxel (2 mM stock when resuspended)
 TXD01
 10 x 100 µl

 Stabilizes microtubules in motor assays
 TXD01
 10 x 100 µl

### ATPase, GTPase, & Phosphatase Biochem Kits™

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) Kinetic quantitation of ATP hydrolysis (Kcat 0.05 to >1.0)	BK051/052	96 assays
<b>CytoPhos™ Phosphate Assay (endpoint assay)</b> Colorimetric assay for ATPases & GTPases (Kcat 0.01 to >1.0)	BK054	1000 assays
GTPase ELIPA™ (enzyme-linked, colorimetric) Kinetic quantitation of GTP hydrolysis (Kcat 0.05 to >1.0)	BK051/052	96 assays
Kinesin ELIPA™ Biochem Kit For real time kinetic and Vmax kinesin ATPase measurements	BK060	96 assays
Kinesin ATPase Endpoint Assay For endpoint measurement of kinesin ATPase activity	BK053	1000 assays
Purine Nucleoside Phosphorylase protein 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**





#### Compound Screening



### **Protein Purification**

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

#### **Example Combinations of Myosins and Actin Filaments**

Cardiac S1 myosin / F-actin

Cardiac S1 myosin / Actin Thin Filament (calcium-sensitive activation) Cardiac Heavy Meromyosin / F-actin

Cardiac Heavy Meromyosin / Actin Thin Filament (calcium-sens. actvn.) Skeletal S1 myosin / F-actin

Skeletal S1 myosin / Actin Thin Filament (calcium-sensitive activation)

Inquire for other combinations not shown here, use the technical support e-mail tservice@cytoskeleton.com.

#### **Combinations of Kinesin/Dyneins and Microtubules**

Eg5 / microtubules CenPE / microtubules Kinesin HC / microtubules Dynein / microtubules MKLP1 / microtubules

MKLP2 / microtubules Any motor from the opposite page or from your own labs can be configured into the core screening format.



Legend: Inhibitors of Guanine Exchange Factors (GEFs), which target small G-proteins such as Ras and Rho, are identified in a kinetic assay with either On or Off rates of GTP or GDP, or by measuring the equilibrium of the nucleotides to indicate a preference for GDP (inactive) or GTP (active).

#### **Example Combinations of GEFs and Small G-Proteins**

SOS1 / K-Ras4B SOS1 / K-Ras4B G12D mutant SOS1 / K-Ras4B other mutant/s Tiam1 / Rac1 Tiam1 / Rac2 Vav1 / Rac1 Vav2 / Rac1

RasGRF1 / H-Ras RasGRF1 / K-Ras4B RasGRF1 / N-Ras Dbs / RhoA Dbs / Cdc42 Inquire for other combinations not shown here, use the technical support e-mail tservice@cytoskeleton.com.

For more information about Protein Purification Services please visit www.cytoskeleton.com/custom-services

Used for measuring protein in:

 Serum Samples **Detergent Compatibility** 

Triton X-100

• Tween 20

NP40 or Igepal

SDS (ADV02 only)

Color signal develops in 1 min

• OD is read at 600 nm with both reagents

Cell Extracts in Detergent Buffers

**Purified Proteins and Antibodies** High Protein Concentration Solutions

Low Protein Concentration (ADV01 only)

#### Protein Assay Reagents

Since 1997, Cytoskeleton has been providing two standard protein assays that enable rapid, accurate, and detergent-compatible measurement of proteins in solution. Every batch is rigorously monitored and quality controlled for excellent batch to batch



matching. The Advanced Protein Assay measures many different proteins with the same signal generation, and has very low detection capability (i.e., 1 µg/ml), whereas Precision Red is useful for measuring protein in cell extracts presented in detergent buffers.

> Legend: ADV02 was used to measure four solutions of purified proteins, optical density was read at 600 nm. For both assays the color signal is developed in I min.

Description	Cat. #	Amount
Advanced Protein Assay™ (5X stock reagent)	ADV01-A	1 x 500 ml
Quantitates protein in the 0.025 - 1.0 mg/ml range	ADV01-B	3 x 500 ml
Precision Red <sup>™</sup> Advanced Protein Assay (1X stock reagent)	ADV02-A	1 x 500 ml
Ouantitates protein in the 0.25-50 mg/ml range	ADV02-B	3 x 500 ml



## **Protein Assay Reagent**

#### Ordering information for USA:

Off Rate GEF

Time(sec.) 600 800

117.10.1 001-6u:

Online - cytoskeleton.com Phone - 303.322.2254 Fax - 303.322.2257 Cytoskeleton, Inc. 1830 S. Acoma St., Denver, CO 80223, USA. International Customers Locate your nearest distributor at: cytoskeleton.com/distributors



## New GTP Exchange Proteins and Assays

SOS1 • Ras-GRF • Tiam1 • Vav1 • Vav2 • ARNO Reliable, pure, and biologically active GEFs, small G-proteins, and assays

## Spirochrome<sup>™</sup> Live Cell Imaging Probes

- SiR-Tubulin Kit
- SiR-Actin Kit
- Cytoskeleton Kit
- SiR-Lysosome Kit
- SiR-DNA Kit
- NEW Far-red Probes

Contact Tebu-Bio to order Spirochrome<sup>™</sup> products in Europe.



Save on Bulk Purchases cytoskeleton.com/bulk

cytoskeleton.com