MOTIF VATIONS

ACTIVE MOTIF®

Tools to Analyze Cellular Function

THE NEWSLETTER OF ACTIVE MOTIF — September 2006 • volume 7 • number 3

Specific, Flexible Labeling of Proteins

Active Motif's LigandLink™* Universal Labeling technology is an innovative tool for the specific labeling of proteins in living cells. With LigandLink, it is now possible to create a single fusion protein that can be labeled with a variety of tags, making it ideal for use in FRET and localization studies.

Improved localization studies

The ability to label proteins in living cells is key to understanding the dynamics and functions of proteins. Classically, these studies have been performed by fusing a gene of interest to a fluorescent protein, such as green fluorescent protein (GFP), and transfecting the construct into cells. These approaches have significantly expanded our understanding of protein function within the cell.

However, protein function is often regulated via protein-protein interactions,

continued on page 6

INSIDE THIS ISSUE

MethylDetector™ – Efficient DNA Methylation Analysis

Active Motif's MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by combining optimized reagents for performing DNA conversion with time-saving DNA purification columns, along with positive control PCR primers that help you validate your results.

Proven controls ensure success

DNA methylation analysis typically involves treating DNA with bisulfite. This converts unmethylated cytosines to uracils while leaving methylated cytosines unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, because bisulfite conversion can be technically challenging, MethylDetector provides positive control PCR primers that are specific for bisulfite-converted DNA. This enables you to confirm that the conversion step was successful before beginning costly sample analysis, saving you time, effort and materials (Figure 1).

Advantages

- **Flexible** – use high G/C content sequences and uncut DNA
- **Reproducible** – 99% conversion efficiency
- **Efficient** – reagents and protocol with proven controls
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

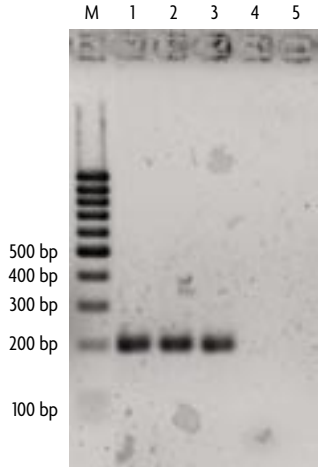

Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes 1-3) and compared to an unconverted DNA control (Lane 5) and to a no DNA control (Lane 4). The presence of PCR product in only the converted samples demonstrates the conversion efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

New: Simple, Efficient Study of Protein SUMOylation

The post-translational modification known as SUMOylation has been shown to be a factor in an increasing number of biological processes. In response, Active Motif developed its SUMOLink™ Kits to fill the need for fast, efficient and robust assays to detect and generate SUMOylated proteins *in vitro*.

SUMOylation is now widely studied because it orchestrates a diverse array of effects on many different biological processes including chromosomal organization and function, DNA repair, nuclear transport and signal transduction.

SUMOLink Kits provide a simple, yet effective method for performing *in vitro* SUMO (small ubiquitin-like modifier) conjugation and detecting SUMOylated proteins. With SUMOLink, all required reagents are provided, including positive control p53 protein and antibody, which help to ensure your success.

"The positive control protein and antibody supplied in SUMOLink Kits help ensure the accuracy of your *in vitro* SUMOylation assays."

The SUMOLink method

SUMOLink Kits are very easy to use. Simply add the assay components to a microcentrifuge tube with your protein of interest and incubate for 3 hours. To aid in result analysis, SUMOLink Kits contain both wild-type and mutated SUMO proteins. After incubation, the wild-type and mutated reactions are stopped and the samples analyzed by Western blot. Antibodies to p53 and either SUMO-1 or SUMO-2/3 are provided so you can monitor the *in vitro* SUMOylation of your protein of interest compared to the positive control p53 protein (Figure 1).

SUMOLink applications

- Investigating SUMOylation's effects on transcription factor activity
- Understanding the role of SUMOylation in the regulation of cellular processes
- Identifying novel proteins that are targets for SUMO proteins

SUMOLink advantages

- Simple, effective method
- Positive control p53 protein and antibody to ensure success
- Wild-type and mutated SUMO proteins are provided
- Versatile – works with recombinant proteins and cell extracts
- All included antibodies react with human, mouse and rat samples

Choose from SUMO-1 or SUMO-2/3

SUMOLink Kits are available for either SUMO-1 detection/conjugation or SUMO-2/3 detection/conjugation. For complete information, visit our website at www.activemotif.com/sumolink.

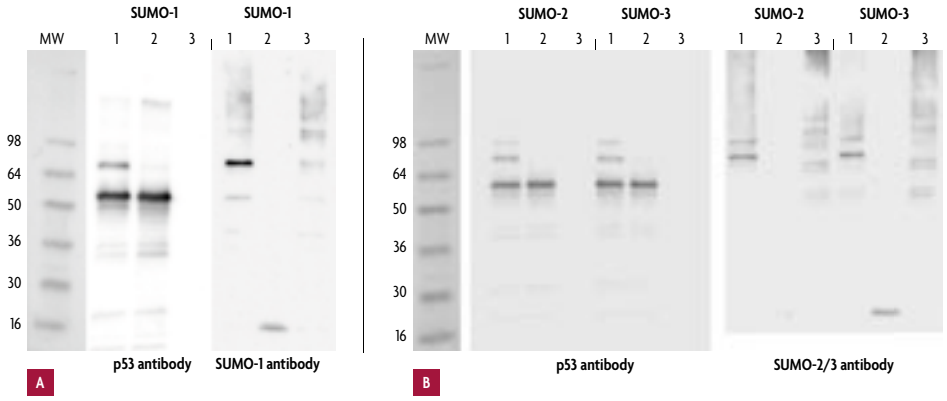

Figure 1: Specific protein labeling using SUMOLink.
Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1, SUMO-2 and SUMO-3 proteins. A. SUMO-1 analysis: Western blots incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution); B. SUMO-2/3 analysis: Western blots incubated with p53 antibody (1:5000 dilution) and SUMO-2/3 antibody (1:4000 dilution).

Lane 1: Wild-type SUMO protein conjugation reaction.
Lane 2: Mutated SUMO protein conjugation reaction.
Lane 3: No p53 control protein used in the conjugation reaction.

Product	Format	Catalog No.
SUMOLink™ SUMO-1 Kit	20 rxns	40120
SUMOLink™ SUMO-2/3 Kit	20 rxns	40220

New: FACE™ – Cell-based ELISAs for EGFR and HSP27

To help scientists keep pace with the ever-changing face of signal transduction pathways, Active Motif continues its development of fast, efficient and robust assays to measure protein phosphorylation with further additions to the Fast Activated Cell-Based ELISA (FACE™) Kit family of products.

FACE Kits provide a simple, efficient, cell-based method to monitor proteins activated by phosphorylation. FACE ELISAs are 96-well, high-throughput assays that do not require cell extracts, electrophoresis or membrane blotting. Unlike Western blotting, FACE enables modification-specific analysis inside the cell. This eliminates the need to perform cumbersome, low-throughput cell extractions, saving you time and money.

The FACE method

In FACE, cells are cultured as desired in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed, which preserves activation-specific protein modifications. Each well is then incubated with a primary antibody specific for the protein modification of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible. FACE Kits also contain a primary antibody for the native non-modified protein, so that you can monitor both native and activated protein levels in the same experiment (Figure 1).

Reproducible, scaleable assay

Typically, phospho-specific analyses are performed on a small number of samples because classical methods, such as Western blot and in-gel kinase assays, are labor intensive and time consuming. This forces you to run multiple experiments on different days and then compare the results. However, as the variability of these methods is high and the induction of phosphorylation is low, this can lead to statistically poor data. Fortunately, FACE Kits make running more samples as simple as adding an extra tip to your multi-channel pipettor. And, because FACE is highly reproducible, you'll get statistically relevant results.

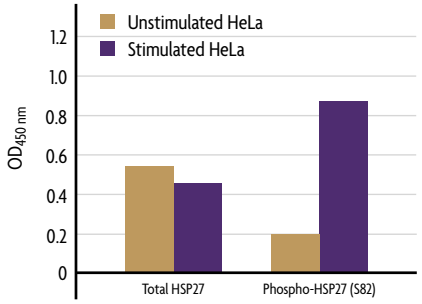

Figure 1: Monitoring total- and phospho-HSP27 using FACE. The FACE HSP27 Kit was used to monitor total and phosphorylated HSP27 (S82) contained within untreated or Anisomycin-treated (25 µg/ml for 30 min) HeLa cells.

FACE advantages

- **Cell-based method** – no need for extracts, gels or membrane blotting
- **Fast** – requires less than 2 hours of hands on time
- **Flexible** – use suspension or adherent cells
- **Total & phospho antibodies** – compare both phosphorylated and native protein levels in the same kit
- **Quantitative results**
- **Versatile** – adaptable for single reactions and high-throughput automation

Latest additions to the FACE line

FACE Kits are now available for monitoring activation of phosphorylation on Tyr845 of EGFR as well as for the detection of phosphorylation of Ser82 on HSP27.

A variety of kits to choose from

FACE Kits are available for many kinases and receptors, as indicated in the table below. All kits are conveniently provided in both colorimetric and ultra-sensitive chemiluminescent formats. For more information about the FACE Kits, please visit www.activemotif.com/face.

FACE™ Product Line			
FACE™ AKT (S473)	FACE™ EGFR (Y992)	FACE™ GSK3β (S9)	FACE™ PI3 Kinase p85
FACE™ ATF-2 (T71)	FACE™ EGFR (Y1173)	FACE™ HSP27 (S82)	FACE™ STAT2 (Y689)
FACE™ Bad (S112)	FACE™ ErbB-2 (Y877)	FACE™ JAK1 (Y1022/Y1023)	FACE™ STAT4 (Y693)
FACE™ c-Jun (S63)	FACE™ ErbB-2 (Y1248)	FACE™ JNK (T183/Y185)	FACE™ STAT6 (Y641)
FACE™ c-Jun (S73)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)	FACE™ MEK1/2 (S217/S221)	
FACE™ c-Src (Y418)	FACE™ FAK (Y397)	FACE™ NFκB p65 Profiler (S468 & S536)	
FACE™ EGFR (Y845)	FACE™ FKHR (T24)	FACE™ p38 (T180/Y182)	

Complete ChIP-IT™ Kits Simplify Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a powerful, yet technically challenging tool used to study protein/DNA interactions. Active Motif's ChIP-IT™ Kits make your ChIP more successful by combining nearly everything you need in a single kit, including positive control antibodies and primers, DNA purification columns and a comprehensive protocol.

The ChIP method

In ChIP, intact cells are fixed using formaldehyde, which cross-links and preserves protein/DNA interactions. The DNA is then sheared into small, uniform fragments. The protein/DNA complexes are then immunoprecipitated using antibodies directed against the DNA-binding protein(s) of interest. Following immunoprecipitation, the cross-links are reversed and DNA fragments are purified and screened to determine which gene, or group of genes, was bound by the protein of interest (Figure 1).

ChIP-IT advantages

- Easy to use – all critical buffers, Protein G beads and DNA purification columns are provided
- No need to optimize reagents and protocol
- Your choice of enzymatic or sonication shearing
- Direct measurement of transcription factor/DNA interactions or histone modifications

Choose enzymatic or sonication shearing

The key to a successful ChIP experiment begins with correctly shearing the DNA into 200-1000 bp fragments. This can be achieved by using one of two methods: enzymatic digestion or sonication. Sonication shearing is an effective method for shearing DNA but can be difficult to optimize due to complications from overheating, emulsification and dependence on sonicator type. In contrast, enzymatic digestion can be used to quickly and easily digest DNA into fragments suitable for ChIP. And, as digestion is dependent only on time and temperature, very little optimization is required. For your convenience, ChIP-IT Kits are available with your choice of either sonication or enzymatic shearing.

Coming soon – ChIP-IT Express Kits

Our soon-to-be released ChIP-IT Express Kits streamline the ChIP procedure, even eliminating DNA purification, making it possible to perform ChIP in just 1 day. Call us or visit our website to find out more.

Start your ChIP today

Active Motif's ChIP-IT Kits provide you with everything you need to make your ChIP experiments easier and more reproducible. For more information on ChIP-IT visit www.activemotif.com/chip.

"ChIP-IT makes performing ChIP with transcription factors more successful by combining all of the critical components you need in a single kit."

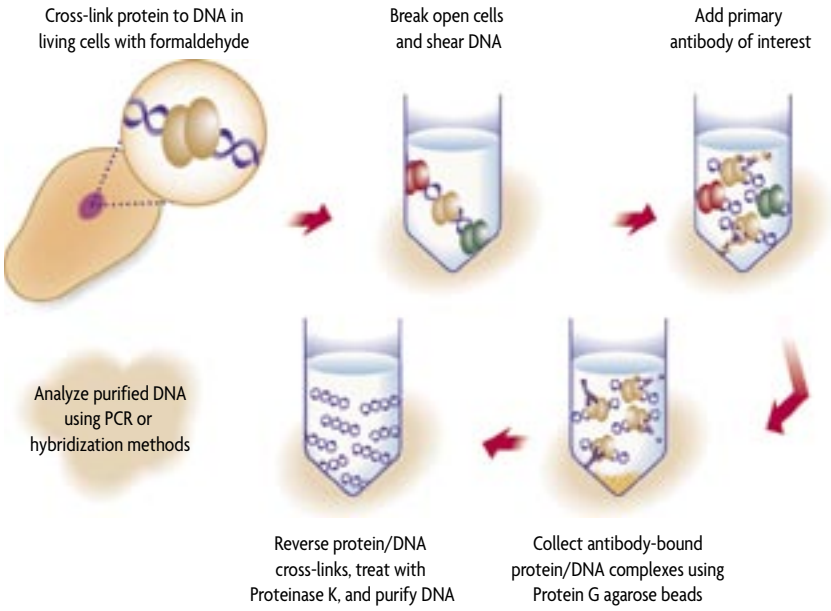

Figure 1: Flow chart of chromatin immunoprecipitation.
In ChIP, protein/DNA interactions are fixed, and the DNA is sheared and precipitated using an antibody. After cross-link reversal, the DNA is purified and screened to determine which genes were bound by the protein of interest.

Product	Format	Catalog No.
ChIP-IT™	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004
ChIP-IT™ Shearing Kit (included in 53001 & 53004)	10 rxns	53002
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic w/o controls	25 rxns	53007
Enzymatic Shearing Kit (included in 53006 & 53007)	10 rxns	53005

Sensitive, Specific Transcription Factor ELISAs

Active Motif's TransAM™ Kits simplify the study of activated transcription factors by combining high sensitivity with convenience in a non-radioactive, ELISA-based assay.

Traditionally, transcription factor activity has been studied using either Electrophoretic Mobility Shift Assays (EMSA), immunoblotting or reporter gene assays. However, these methods are quite time-consuming and, at best, provide only semi-quantitative results. Moreover, use of these methods to determine the activity of multiple transcription factor family members would require the investment of significant amounts of both time and money; in many cases, it may not even be possible.

Simple, quantitative assay

In contrast, Active Motif's TransAM Kits make it straightforward to measure the activity of transcription factors. You can even study multiple members of a transcription factor family in a single experiment that is complete in less than 5 hours. In addition to being fast, the TransAM method is non-radioactive and provides quantitative results (Figure 1). Plus, TransAM Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

"TransAM Kits are sensitive, non-radioactive DNA-binding ELISAs that facilitate the study of transcription factor activation in cell and tissue samples."

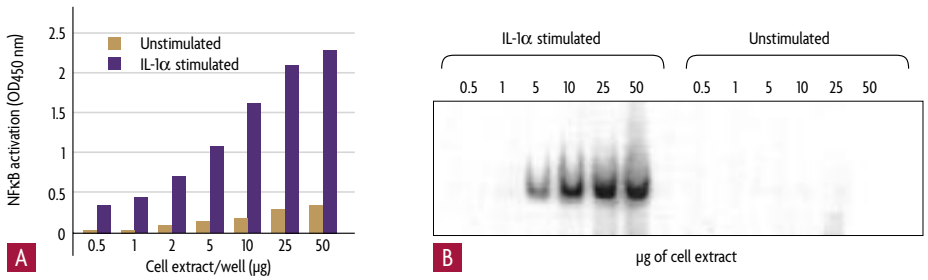

Figure 1: TransAM NFκB is more sensitive than gelshift, and provides more quantitative results.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using either the TransAM NFκB p50 Kit (A) or gel retardation (B).

Flexi or Original format

The Original TransAM Kits offer a fast, non-radioactive alternative to gelshift by providing a 96-well plate that is precoated with oligonucleotide that contains a consensus-binding site for the factor of interest. Activated transcription factor binds the oligo and is quantified on a spectrophotometer using the supplied antibodies and developing reagent. This format is convenient for measuring binding at the consensus site, but does not enable you to study alternative sites. For this reason, we developed TransAM Flexi Kits; they contain all of the optimized reagents provided in the Original kits, including antibodies, but give you the flexibility to immobilize any oligo in the 96-well plate.

TransAM advantages

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift (100-fold with Chemi Kits)
- Simultaneous profiling of multiple family members
- Assay both cell and tissue samples

Try sensitive, quantitative ELISAs

TransAM Kits make it simple to quantify activated transcription factors. Please give us a call or visit our website to learn more about the TransAM product line.

TransAM™ Product Line				
TransAM™ AP-1 Family	TransAM™ AML-3/Runx2	TransAM™ CREB & pCREB	TransAM™ MEF2	TransAM™ Oct-4
TransAM™ GATA Family	TransAM™ AP-1 c-Fos	TransAM™ Elk-1	TransAM™ MyoD	TransAM™ p53
TransAM™ HNF Family	TransAM™ AP-1 c-Jun	TransAM™ ER	TransAM™ NF-YA	TransAM™ PPARγ
TransAM™ MAPK Family	TransAM™ AP-1 FosB	TransAM™ FKHR (FOXO1)	TransAM™ NFATc1	TransAM™ Sp1 & Sp1/Sp3
TransAM™ Flexi NFκB Family	TransAM™ AP-1 JunD	TransAM™ GATA-4	TransAM™ Flexi NFκB p50	TransAM™ STAT3
TransAM™ NFκB Family	TransAM™ ATF-2	TransAM™ GR	TransAM™ NFκB p50*	
TransAM™ STAT Family	TransAM™ c-Myc	TransAM™ HIF-1	TransAM™ Flexi NFκB p65	
TransAM™ AML-1/Runx1	TransAM™ C/EBP α/β	TransAM™ HNF-1	TransAM™ NFκB p65*	

* The Original TransAM NFκB p50 & p65 Kits are offered in both colorimetric and chemiluminescent formats. TransAM Chemi Kits require the use of a luminometer.

continued from page 1 — Specific, Flexible Labeling of Proteins

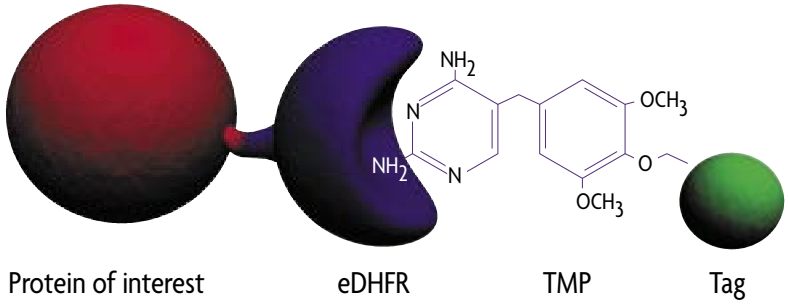

Figure 1: Specific protein labeling using LigandLink.
The gene of interest is cloned into pLL-1 in frame with the vector's *E. coli* dihydrofolate reductase (eDHFR) gene. After transfection into cells, the protein of interest is expressed as a fusion to eDHFR. Addition of cell-permeable LigandLink Label to the medium results in rapid, specific binding of the label's trimethoprim (TMP) ligand by eDHFR on the fusion protein.

creating the need for multiple labeling systems. Using conventional approaches, you would need to clone a series of constructs with your gene of interest fused to different fluorescent proteins. This has made such studies time-consuming and labor intensive. In contrast, LigandLink makes it possible to create a single clone that can be labeled with different tags. This makes LigandLink ideal for FRET and localization, where multiple labeling colors are required.

Why use LigandLink?

- **Spend less time on cloning and clone validation** – clone only once, then obtain different functionalities by using different LigandLink Labels
- **Small label** – eDHFR is much smaller than GFP, so is unlikely to interfere with protein functions/interactions
- **High specificity** – TMP is an antibiotic designed to bind eDHFR, but not interact with mammalian proteins
- **Superior fluorescence** – synthetic dyes have improved spectral properties vs. naturally fluorescent proteins
- **Label your protein in living cells** – LigandLink Labels are cell permeable

The LigandLink method

In the LigandLink method, the gene of interest is cloned in frame with the gene for *E. coli* dihydrofolate reductase (eDHFR) in the pLL-1 vector. This vector has a CMV promoter for high-level expression of eDHFR fusion proteins, with Neomycin for selection of stable cell lines. The vector is transfected into mammalian cells and used to express the fusion protein. Twenty-four hours post-transfection, the protein of interest can be labeled simply by adding the LigandLink Label of choice to the cell medium (Figure 1). Depending on the cell type and the label used, cells can be imaged in as little as 10 minutes.

Pre-made translocation vectors

The LigandLink Universal Labeling technology is ideal for studying translocation events because of the highly permeable nature of the TMP ligand and the simple way in which proteins are labeled in *in vivo* environments (Figure 2). To make it even easier, we have already prepared a line of pre-made vectors containing transcription factors such as NFκB, STAT and p53 that are ready to transfect into the mammalian cell line of your choice.

Try LigandLink today

LigandLink will improve the way you label proteins in living cells. The highly specific and stable LigandLink tags are ideal for use in FRET and localization research because once a protein of interest is expressed as a LigandLink fusion, you can easily change the properties of its tag by adding a different LigandLink label to the medium. Find out more about the new LigandLink system by visiting www.activemotif.com.

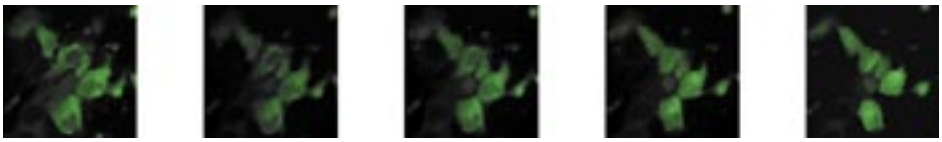

Figure 2: Labeling of NFκB p65 by LigandLink Fluorescein.
U2OS cells were transfected with the pLL-1-NFκB p65 vector. Two days later, the cells were stimulated by TPA (50 ng/ml) and CI (0.5 µM) and labeled by addition of LigandLink Fluorescein Label to the media. Prior to stimulation, NFκB p65 is located in the cytoplasm of the cell. Upon activation, the p65 protein translocates to the nucleus.

Product	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-AKT1 Kit	1 kit	34002
LigandLink™ pLL-1-AKT2 Kit	1 kit	34003
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

* Patent pending.

New: Improved Fluorescent Labels for Bioanalysis

Active Motif's Chromeo™ Dyes provide you with an improved tool for fluorescently labeling biomolecules. Their unique spectral properties include excellent photostability, pH tolerance and low cell toxicity, making them ideal for any application.

Excellent fluorescent properties

The Chromeo Dyes – Chromeo 494, Chromeo 546 and Chromeo 642 – exhibit many key features including superior luminescence properties and broad Stokes shifts (Table 1). Chromeo Dyes are also suitable for use with many common excitation sources such as diode lasers, LEDs, tungsten and Xenon arc lamps.

Multiple applications

The Chromeo Dyes are available in several formats including as reactive NHS-esters that can be used to label amino groups such as antibodies (Figure 1). They are also available as carboxylic acids and as biotin and streptavidin conjugates. In addition, Chromeo 494 has a very large Stokes shift of 124 nm, making it an ideal partner for multiplexing with other, shortshifted 488-excitable dyes.

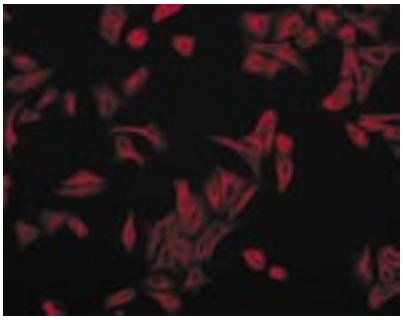

Figure 1: Chromeo 546 conjugated secondary antibody. Chromeo 546 was conjugated to anti-rabbit secondary antibody and used to label tubulin in U2OS cells.

Advantages

- Convenient spectral properties
- High quantum yields
- pH insensitive
- Excellent photostability
- Available as reactive NHS-Esters, carboxylic acid, biotin or streptavidin conjugates

Visit the Active Motif Chromeon website

As a leader in providing fluorescent tools for bioanalysis, Active Motif Chromeon also offers several other dyes to meet your research needs, including RuLabels and Py-Dyes. RuLabels are phosphorescent ruthenium probes that emit strong polarized light with a long decay time, which enhances sensitivity and reduces background. The Py-Dyes possess unique physical and spectral properties that make conjugation simple, while greatly reducing background. To find out more about Active Motif Chromeon products visit www.chromeon.com.

Dye	Absorption	Emission	ε L/(mol-cm)	Quantum Yield (%)*	Stokes Shift
Chromeo™ 494	494	628	20,000	25	124 nm
Chromeo™ 546	545	561	96,800	10	16 nm
Chromeo™ 642	642	660	180,000	15	18 nm

Table 1: Chromeo Dye properties. (* when conjugated to BSA)

Product	Format	Catalog No.
Chromeo™ 494 Carboxylic Acid	1 mg 5 mg	15110 16110
Chromeo™ 494 NHS-Ester	1 mg 5 mg	15111 16111
Chromeo™ 494 Biotin	1 mg 5 mg	15112 16112
Chromeo™ 494 Streptavidin	1 mg 5 mg	15113 16113
Chromeo™ 546 Carboxylic Acid	1 mg 5 mg	15210 16210
Chromeo™ 546 NHS-Ester	1 mg 5 mg	15211 16211
Chromeo™ 546 Biotin	1 mg 5 mg	15212 16212
Chromeo™ 546 Streptavidin	1 mg 5 mg	15213 16213
Chromeo™ 642 Carboxylic Acid	1 mg 5 mg	15310 16310
Chromeo™ 642 NHS-Ester	1 mg 5 mg	15311 16311
Chromeo™ 642 Biotin	1 mg 5 mg	15312 16312
Chromeo™ 642 Streptavidin	1 mg 5 mg	15313 16313

New: Accurate Cell-by-Cell Fluorescent Viability Assay

The ToxCount™ Cell Viability Assay provides reagents for a simple two-color fluorescent cell viability assay. Calcein AM is used to detect live cells, while ethidium homodimer (EthD-1) detects dead cells within a population.

The ToxCount assay is ideal for the rapid screening of drug and small molecule libraries for cytotoxic effects. ToxCount is particularly applicable to high-content, cell-by-cell analysis (Figure 1) using the BlueShift Biotechnologies IsoCyte™ laser scanner (www.blueshiftbiotech.com).

Save time and money

ToxCount is less expensive, safer and faster than other cell viability assays such as 51Cr-release, LDH release and trypan blue exclusion, and can be used on multiple fluorescence detection platforms including multi-well plate scanners, fluorescence microscopy and flow cytometry.

ToxCount advantages

- Accurate and reproducible
- Quick and easy assay
- Non-toxic reagents
- Economical
- High-throughput compatible

"Use ToxCount for accurate counts of live/dead cells in a population."

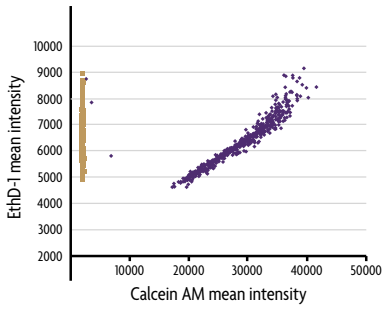

Figure 1: Analysis of ToxCount data using the IsoCyte. The graph depicted above illustrates the mean intensity ratio for EthD-1 and calcein AM staining in each cell from two separate wells. One well was treated with saponin (copper dots), resulting in cell death and, therefore, staining with EthD-1. The second well was untreated (purple dots), resulting in calcein AM staining. Three cells in the untreated well were unhealthy, so were stained by the EthD-1.

Product	Format	Catalog No.
ToxCount™	20 x 96 rxns	18010

8 U.S.
Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351
Email: sales@activemotif.com

THE NEWSLETTER OF ACTIVE MOTIF — September 2006 • volume 7 • number 3

ACTIVE MOTIF®
Tools to Analyze Cellular Function

PRSRT STD
US POSTAGE
PAID
PERMIT #236
92054