

Novagen®

WideScreen™  
EpiTag™ ERK Pathway Assays  
for the Luminex® xMAP® Technology platform

Featuring Epitome Biosystems, Inc.  
EpiTag™ Technology



# WideScreen™ EpiTag™ Assays

## ERK Pathway

for the Luminex® xMAP® Technology platform

The Novagen® WideScreen™ Group has developed immuno-assay panels using a novel, powerful assay platform for the multiplex quantitation of phosphorylated proteins. WideScreen EpiTag™ Assays for the bead-based xMAP® platform utilize EpiTag™ Technology, licensed from Epitome Biosystems, Inc., which detects protein- and phosphoprotein-fragments rather than the traditional detection of intact proteins.

Protein fragments are released by digestion with site-specific proteases. Antibody pairs are generated to unique amino acid sequences, or EpiTag™ Sequences, on these fragments. This approach provides a high degree of specificity and the ability to multiplex assays for a protein's total and phosphorylated levels without cross-reactivity. In addition, the assay can accurately measure concentrations of phosphorylated target proteins as synthetic phosphopeptides are used to generate standard curves.



WideScreen Group,  
Madison, U.S.A.

The Novagen® WideScreen™ Group, an international interdisciplinary team of assay development scientists, was assembled to introduce the next generation of multiplex immuno-assays. After extensive R&D, the first wave of unique assay panels created by the Group are available for the bead-based Luminex® xMAP® Technology platform.

# WideScreen™ EpiTag™ Assays Principle\*

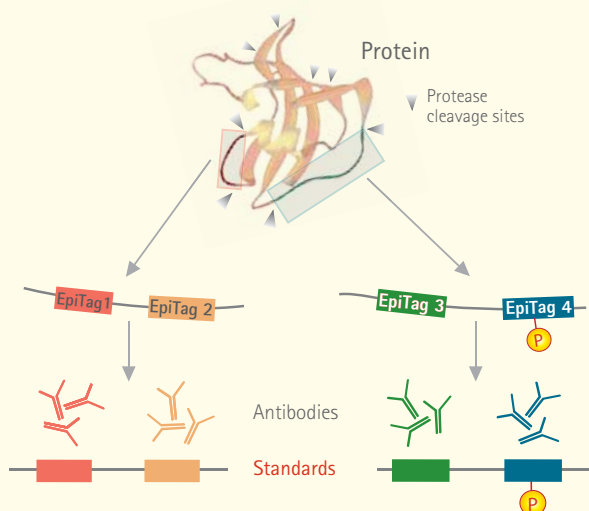
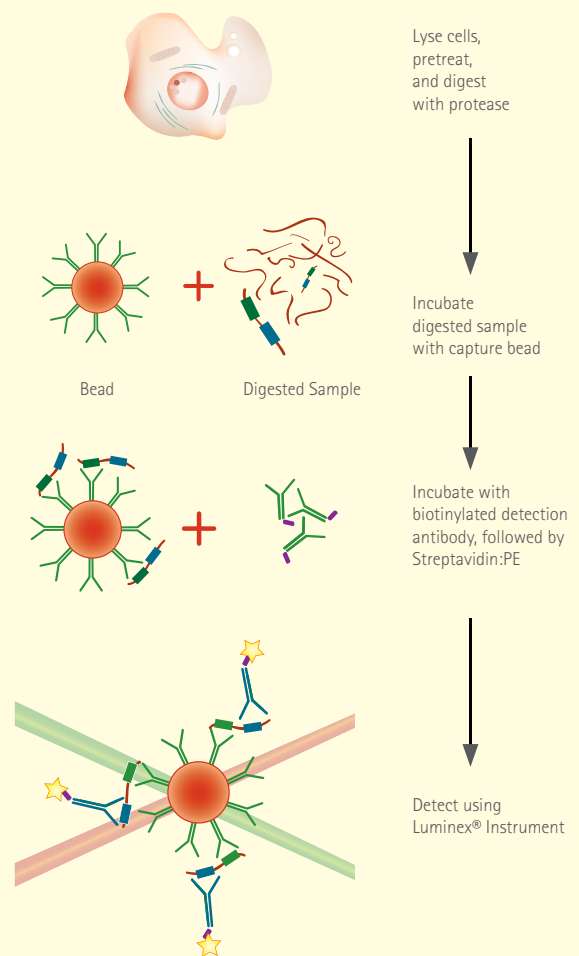
Cells or tissues are lysed and the extracted proteins are digested with a specific protease to release proteolytic fragments. The digested lysate is then used in a standard-format xMAP® Assay. A single sample preparation procedure is used for all WideScreen™ EpiTag™ Assays, removing the guesswork out of choosing a lysis buffer. The digested lysates are stable and can be frozen and thawed several times without significant loss of signal, providing an unparalleled degree of workflow flexibility.

Targeting different fragments from the same protein allows both total- and phospho-assays to be run together. This is a significant advantage over current assays where total- and phospho-assays cannot be multiplexed and are run in separate wells.

The use of synthetic peptides and phosphopeptides as standards to generate standard curves allows direct comparisons of total- and phosphoprotein-concentrations in cell lysates.

\* Due to the unique nature of the EpiTag™ Technology and sample preparation, the EpiTag™ buffers and bead kits are **incompatible** with other Novagen® brand bead-based assay buffers and bead kits or those from other companies.

## EpiTag™ xMAP® Assay



## Epitome Biosystems EpiTag™ Technology

Advanced bioinformatics is employed to identify proteolytic fragments of target proteins that contain two or more unique regions, or EpiTag™ Sequences. Antibodies against the EpiTag Sequences are used as capture and detection reagents to form sandwich immunoassays. Synthetic peptides that represent the targeted fragment are used as standards.

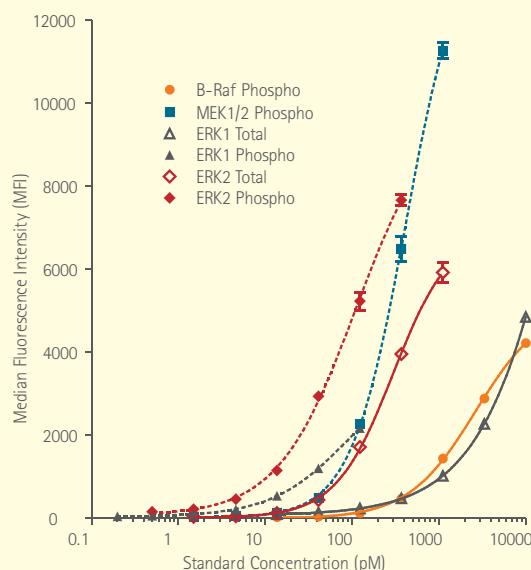
For more details, visit [www.epitomebiosystems.com](http://www.epitomebiosystems.com)

EpiTag™ Technology and Assays are offered under limited, non-transferable license from Epitome Biosystems, Inc.

# WideScreen™ EpiTag™ Assays

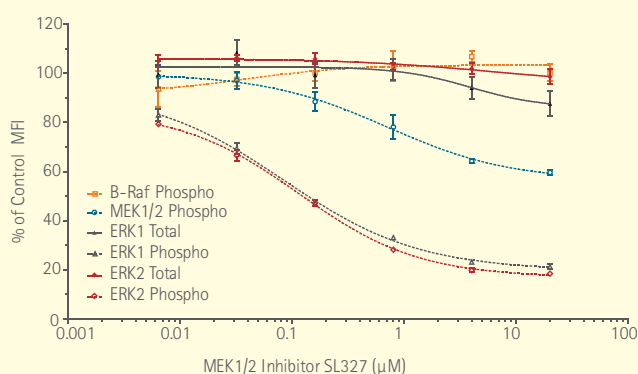
## ERK Pathway

The first WideScreen™ EpiTag™ Assays have been developed to detect key signaling events in the ERK pathway from both human and mouse samples. ERK1 and ERK2 are serine/threonine kinases expressed broadly in tissues and various cell lines. ERKs are activated through their phosphorylation by the MAPK/ERK kinases MEK1 and MEK2. Once activated, ERK1 and ERK2 can phosphorylate many different proteins including cytoskeletal proteins, translation regulators, and transcription factors. ERK pathway protein levels and phosphorylation are deregulated in many types of cancer and are considered strong candidates for the development of therapeutic compounds.



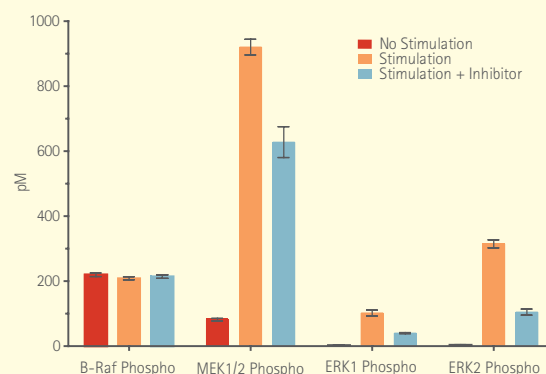
### Standard Curve Multiplex

Three-fold serial dilutions of peptide and phosphopeptide standards were measured using the WideScreen™ EpiTag™ ERK Pathway Panel I Complete Kit. The EpiTag™ ERK Pathway Standards correspond to proteolytic fragments (1:1 ratio with target protein) generated during cell lysate digestion, and thus the standard curves can be used for molar quantitation of target proteins.



### Specific Inhibition of ERK Phosphorylation after treatment with a MEK Inhibitor

U87 cells were cultured in 96-well plates, serum-starved overnight, and treated with the MEK1/2 inhibitor SL327 at different concentrations for 1 h. Following rh-EGF stimulation (5 min), cell lysates were prepared, digested, and assayed using the listed EpiTag™ Bead Kits. Data shown are averages from triplicate culture wells ( $\pm$  SEM), expressed as % of uninhibited controls. Total protein levels are unaffected by inhibitor treatment, whereas ERK1 and ERK2 phosphorylation is inhibited with increased concentrations of inhibitor. The inhibitor dose response correlates closely with the expected SL327  $IC_{50}$  of ~200 nM.



### Stimulation and Inhibition of ERK Pathway Target Phosphorylation

Serum-starved A431 cells were left untreated, stimulated with rh-EGF (100 ng/ml, 5 min, Calbiochem Cat. No. 324831), or stimulated with rh-EGF after 1 hr pretreatment with 4 mM of the MEK1/2 inhibitor SL327 (Calbiochem Cat. No. 444939). EpiTag™ ERK Pathway Bead Kits were used to obtain quantitative measurements of the phosphoproteins shown (averages from triplicate culture wells  $\pm$  SEM). MEK and ERK proteins are phosphorylated upon stimulation, whereas B-Raf phosphorylation at S446 is reported to be constitutive. The MEK1/2 inhibitor SL327 strongly inhibits the phosphorylation of downstream ERK proteins.

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## Current xMAP® Assays

## NEW WideScreen™ EpiTag™ Assays

Quantitation of phosphoproteins	Partially phosphorylated protein standards (i.e. "units") Standards often not provided	Synthetic phosphopeptide standards (absolute quantitation) Standards always provided
Specificity	Screen many antibodies Difficult to discriminate closely related proteins	Specificity by bioinformatics Can discriminate related proteins
Multiplex	Total- and phospho-assays cannot be multiplexed	Total- and phospho-assays can be multiplexed
Sample Preparation	Target-specific lysis buffers Unstable lysates	One lysis buffer Procedure and lysates are robust Benzonase® Nuclease reduces sample viscosity to enhance assay performance

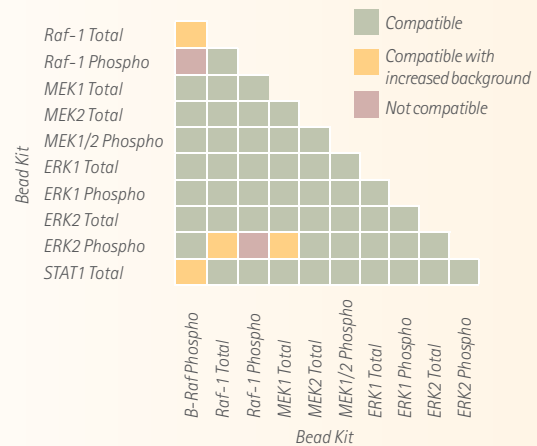
## Flexible Configuration

The WideScreen™ EpiTag™ ERK Pathway Assays are designed to be flexible. The assays are available as Complete Kits which contain all lysis, wash and digest buffers, premixed beads, and detection cocktails needed to run an assay. The components of the Complete Kits are also available separately, enabling greater cell culture and processing options.

For even greater flexibility the assays are available in two premixes and as individual bead kits:

- **Premixed WideScreen EpiTag ERK Panels I and II** for the detection of two groups of 6 ERK pathway analytes.
- **Individual bead kits** allowing the creation of custom multiplexes\*

### Compatibility Chart



### WideScreen EpiTag™ ERK Pathway Panel I Complete Kit

#### EpiTag™ ERK Panel I:

B-Raf (pS446)  
MEK1/2 (pS217, pS221/ pS222, pS226)  
ERK1 Total  
ERK1 (pT202/pY204)  
ERK2 Total  
ERK2 (pT185/pY187)

EpiTag™ ERK Pathway Standards Mix

EpiTag™ Cell Lysis Kit<sup>1</sup>

EpiTag™ Digest Kit<sup>2</sup>

WideScreen Reagent Kit<sup>3</sup>

### WideScreen EpiTag ERK Pathway Panel II Complete Kit

#### EpiTag ERK Panel II:

MEK1 Total  
MEK2 Total  
MEK1/2 (pS217, pS221/ pS222, pS226)  
Raf1 Total  
Raf1 (pS338)  
STAT1 Total

EpiTag ERK Pathway Standards Mix

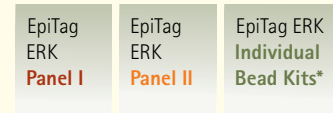
EpiTag Cell Lysis Kit

EpiTag Digest Kit

WideScreen Reagent Kit

### WideScreen EpiTag ERK Pathway User-defined assay

#### Choice of:



EpiTag ERK Pathway Standards Mix

EpiTag Cell Lysis Kit

EpiTag Digest Kit

WideScreen Reagent Kit

For convenience, the EpiTag Cell Lysis Kit, EpiTag Digest Kit, and WideScreen Reagent Kit are also available together as the **EpiTag Combo Reagent Kit**.

\*Please consult the Compatibility Chart to confirm which bead kits can be multiplexed.

<sup>1</sup> EpiTag Cell Lysis Kit includes: Lysis Reagent, Phosphatase Inhibitor Cocktail Set V, Benzonase® Nuclease HC, Purity >99%, 100X TCEP Solution, Aluminum Plate Sealer.

<sup>2</sup> EpiTag Digest Kit includes: α-Iodoacetamide, Endopeptidase Lys-C.

<sup>3</sup> WideScreen Reagent Kit includes: Streptavidin-PE Concentrate, 96-Well Filter Plate and Sealer, 5X Assay Diluent, 10X Wash Buffer.

Product	Components	Size	Cat. No.
WideScreen™ EpiTag™ ERK Pathway Panel I Complete Kit	EpiTag Cell Lysis Kit, EpiTag Digest Kit, WideScreen Reagent Kit, EpiTag ERK Pathway Standards Mix, EpiTag ERK Pathway Panel I. For the detection of B-Raf (pS446), MEK1/2 (pS222/pS226), ERK1 Total, ERK1 (pT202/pY204), ERK2 Total and ERK2 (pT185/pY187)	96 tests	71782-3
WideScreen™ EpiTag™ ERK Pathway Panel II Complete Kit	EpiTag Cell Lysis Kit, EpiTag Digest Kit, WideScreen Reagent Kit, EpiTag ERK Pathway Standards Mix, EpiTag ERK Pathway Panel II. For the detection of Mek1 Total, Mek2 Total, MEK1/2 (pS222/pS226), Raf1 Total, Raf1 (pS338), and STAT1 Total	96 tests	71891-3
EpiTag™ Cell Lysis Kit	Lysis Reagent, Phosphatase Inhibitor Cocktail Set V, Benzonase® Nuclease HC, Purity >99%, 100X TCEP Solution, Aluminum Plate Sealer	1 kit	71784-3
EpiTag™ Digest Kit	α-Iodoacetamide, Endopeptidase Lys-C	1 kit	71785-3
WideScreen™ Reagent Kit	Streptavidin-PE Concentrate, 96-Well Filter Plate and Sealer, 5X Assay Diluent, 10X Wash Buffer	1 kit	71783-3
EpiTag™ Combo Reagent Kit	EpiTag Cell Lysis Kit, EpiTag Digest Kit, WideScreen Reagent Kit	1 set	71794-3
EpiTag™ ERK Pathway Standards Mix		1 vial	71791-3
EpiTag™ ERK Pathway Panel I	ERK Pathway Capture Beads Premix I, ERK Pathway Detection Antibody Premix I. For the detection of B-Raf (pS446), MEK1/2 (pS222/pS226), ERK1 Total, ERK1 (pT202/pY204), ERK2 Total and ERK2 (pT185/pY187)	100 tests	71826-3
EpiTag™ ERK Pathway Panel II	ERK Pathway Capture Beads Premix II, ERK Pathway Detection Antibody Premix II. For the detection of Mek1 Total, Mek2 Total, MEK1/2 (pS222/pS226), Raf1 Total, Raf1 (pS338), and STAT1 Total	100 tests	71890-3
EpiTag™ ERK1 Total Bead Kit	ERK1 Total Capture Beads, ERK1 Total Detection Antibody	100 tests	71829-3
EpiTag™ ERK2 Total Bead Kit	ERK2 Total Capture Beads, ERK2 Total Detection Antibody	100 tests	71830-3
EpiTag™ B-Raf (pS446) Bead Kit	B-Raf (pS446) Capture Beads, B-Raf (pS446) Detection Antibody	100 tests	71831-3
EpiTag™ MEK1/2 (pS217, pS221 / pS222, pS226) Bead Kit	MEK1/2 (pS217, pS221 / pS222, pS226) Capture Beads, MEK1/2 (pS217, pS221 / pS222, pS226) Detection Antibody	100 tests	71832-3
EpiTag™ ERK1 (pT202, pY204) Bead Kit	ERK1 (pT202, pY204) Capture Beads, ERK1 (pT202, pY204) Detection Antibody	100 tests	71833-3
EpiTag™ ERK2 (pT185, pY187) Bead Kit	ERK2 (pT185, pY187) Capture Beads, ERK2 (pT185, pY187) Detection Antibody	100 tests	71834-3
EpiTag™ MEK1 Total Bead Kit	MEK1 Total Capture Beads, MEK1 Total Detection Antibody	100 tests	71835-3
EpiTag™ MEK2 Total Bead Kit	MEK2 Total Capture Beads, MEK2 Total Detection Antibody	100 tests	71836-3
EpiTag™ STAT1 Total Bead Kit	STAT1 Total Capture Beads, STAT1 Total Detection Antibody	100 tests	71837-3
EpiTag™ Raf1 Total Bead Kit	Raf1 Total Capture Beads, Raf1 Total Detection Antibody	100 tests	71838-3
EpiTag™ Raf1 (pS338) Bead Kit	Raf1 (pS338) Capture Beads, Raf1 (pS338) Detection Antibody	100 tests	71839-3

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