



cell signaling solutions

Certificate of Analysis

48 Barn Road • Lake Placid, NY 12946

Technical Support: T: 800 548-7853 • F: 518 523-4513

email: techserv@upstate.com

Sales Department: T: 800 233-3991 • F: 781 890-7738

Licensing Dept.: 800 310-4659

www.upstate.com

Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmates™

(100 Assay Points)

Catalog # 46-602

Lot # 24878A

Components

Beadlyte® Anti-Erk/MAP Kinase 1/2 Beads, Catalog # 42-602, Lot # 24878. One vial containing **125µl** of anti-Erk/MAP Kinase 1/2 rabbit polyclonal IgG conjugated to Luminex® **Bead # 9** at **2000 beads/µl (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-phospho-Erk/MAP Kinase 1/2, Biotin, Catalog # 44-602, Lot # 24878A. One vial containing **125µl** of anti-phospho-Erk/MAP Kinase 1/2 rabbit polyclonal IgG **(20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes human and mouse phosphorylated Erk/MAP Kinase 1/2 (p42/p44 MAPK).

Applications: Optimal antibody pair for detection of phosphorylated Erk/MAP Kinase 1/2. To be used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600).

Storage and Stability: Stable for 1 year at 4°C from date of shipment. Store in the **dark**.

FOR IN VITRO RESEARCH USE ONLY

NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS

DO NOT USE IN HUMANS OR IN ANIMALS

Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmate™ Description

Use: The Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated Erk/MAP Kinase 1/2 (Thr185/Tyr187) in cell lysates using the Luminex® 100™ system. Each Beadmate™ pair is ordered individually and can be combined for simultaneous multiplex analysis of cellular events. The Beadlyte® Cell Signaling Buffer Kit is also ordered separately and consists of a common set of reagents needed for using Beadmates™. The detection assay is a rapid, convenient alternative to Western Blotting and immunoprecipitation procedures. Each kit contains sufficient reagents for 100 individual assays.

Important note: The Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmate™ pair CANNOT be multiplexed with the Beadlyte® Total Erk/MAP Kinase 1/2 Beadmate™ pair (Catalog # 46-609) since it would require a second reporter fluorochrome on one of the antibodies. The current Luminex systems are **not** able to perform two color analysis at this time. For a detailed protocol on Cell Signaling Detection Procedures please see the COA for the Beadlyte® Cell Signaling Buffer Kit available at:

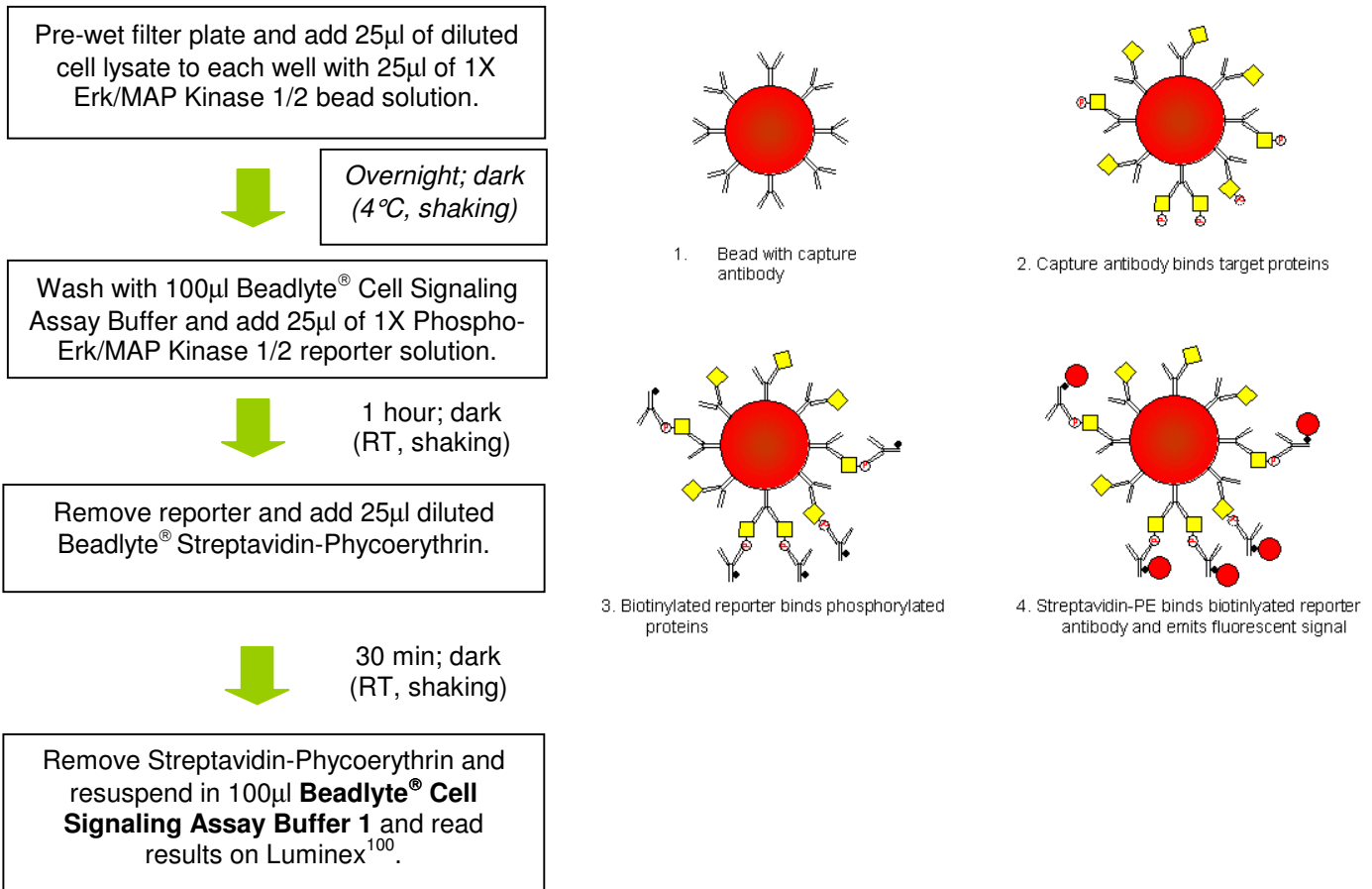
<http://www.upstate.com/img/coa/48-600-24907.pdf>

Other components required but not included as part of kit are:

- Cell lysates or cell extracts harboring protein(s) of interest
- Vortex mixer
- Plate shaker
- Timer
- Variable volume (5-200 μ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex[®] LabMAP[™] System
- Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600)

Detection Protocol Summary

The assay procedure is a simple fluorescent bead-based sandwich immunoassay that is sensitive and easy to perform. A cell lysate or other sample is incubated with beads coupled to an Erk/MAP Kinase 1/2 specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-Erk/MAP Kinase 1/2 specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-Erk/MAP Kinase 1/2 is then quantified using the Luminex¹⁰⁰ LabMAP[™] System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of Erk/MAP Kinase 1/2.



Recommendations for Protocol

Preparation of lysates

For a single plex analysis, Beadlyte[®] Cell Signaling **Lysis Buffer B** is recommended for lysing cells for Phospho-Erk/MAP Kinase 1/2 single plex analysis. This lysate buffer is included in the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600). Refer to the Beadlyte[®] Cell Signaling Buffer Kit COA for a suggested cell lysis protocol at: <http://www.upstate.com/img/coa/48-600-24907.pdf>.

Note: If the cell lysate is to be used in a multiplex assay with phospho-Erk/MAP Kinase 1/2 beads and other Beadmates[™], please refer to the Buffer Selection Table in the Beadlyte[®] Cell Signaling Buffer Kit COA at <http://www.upstate.com/img/coa/48-600-24907.pdf> to select the best Lysis Buffer.

Preparation of Phospho- Erk/MAP Kinase 1/2 Beads and reporter antibodies

For Phospho-Erk/MAP Kinase 1/2 single plex analysis, Beadlyte[®] Cell Signaling **Assay Buffer 1** is recommended for best results (Beadlyte[®] Cell Signaling Buffer Kit, Catalog # 48-600).

Note: If Phospho-Erk/MAP Kinase 1/2 beads are being multiplexed with other Beadmates[™], please refer to the Buffer Selection Table in the Beadlyte[®] Cell Signaling Buffer Kit COA at <http://www.upstate.com/img/coa/48-600-24907.pdf> to select the best Assay Buffer to use.

Phospho- Erk/MAP Kinase 1/2 Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Phospho-Erk/MAP Kinase 1/2	46-602	#9	A	0	0-20	0-20
			B	100	80-100	40-60
			C	0-20	20-40	0

Representative Data:

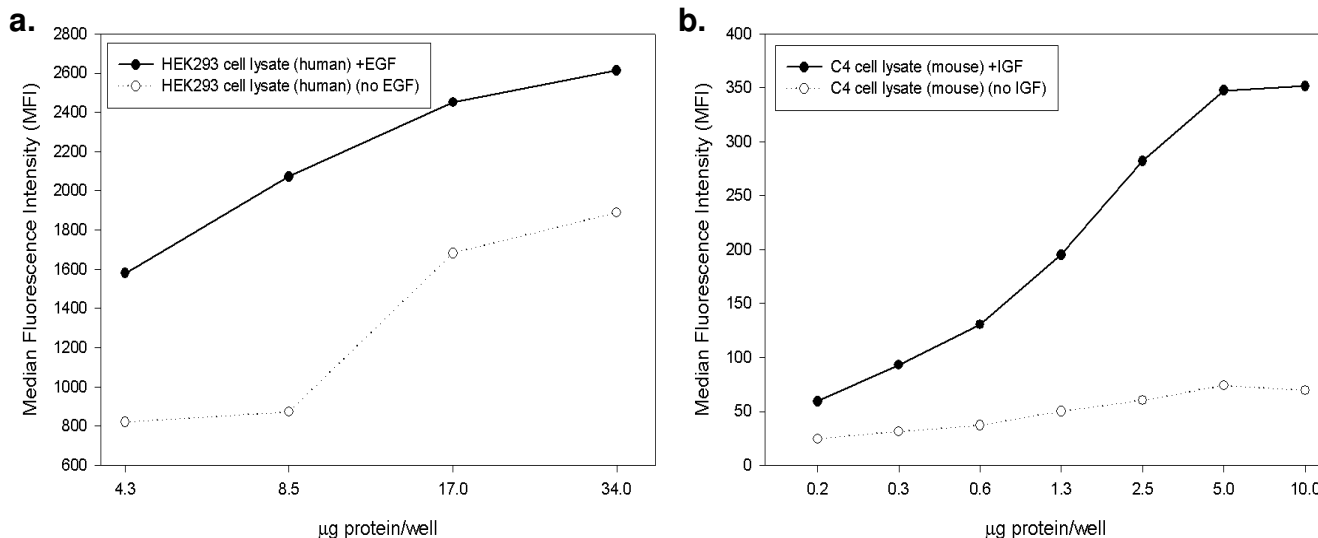
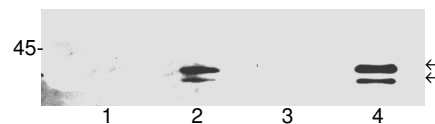


Figure 1. Beadlyte® detection of phosphorylated Erk/MAP Kinase 1/2 proteins in HEK293 cell lysate (a) and C4 cell lysate (b). HEK293 cells (a) were grown to confluence and stimulated with (●) or without (○) 50ng/ml of EGF for 10 minutes. C4 cells (b) were grown to confluence and stimulated with (●) or without (○) 50ng/ml of IGF for 15 minutes. Increasing amounts of cell lysate (lysed in Beadlyte® Cell Signaling Lysis Buffer A with protease inhibitors) were incubated overnight at 4°C with Beadlyte® Anti-Erk/MAP Kinase 1/2 Beads. The Beads were washed and mixed at room temperature with Beadlyte® Anti-phospho-Erk/MAP Kinase 1/2, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex¹⁰⁰ LabMAP™ system.

Figure 2. Western blot detection of phosphorylated Erk/MAP Kinase 1/2 in HEK293 cell lysate and C4 cell lysate. HEK293 cells were grown to confluence and stimulated with or without 50ng/ml of EGF for 10 minutes. C4 cells were grown to confluence and stimulated with or without 50ng/ml of IGF for 15 minutes. 10µg/well of unstimulated HEK293 (lane 1), stimulated HEK293 (lane 2), unstimulated C4 (lane 3) or stimulated C4 (lane 4) cell lysate (lysed in Beadlyte® Cell Signaling Lysis Buffer A with protease inhibitors) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with rabbit anti-phospho-Erk/MAP Kinase 1/2. Blots were incubated with HRP labeled anti-rabbit IgG and visualized via chemiluminescence. Arrows indicate phosphorylated Erk/MAP Kinase 1/2 (42/44kDa).



End-User License Agreement

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex™, you, the customer, acquire the right under Luminex's™ patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex's™ laser based fluorescent analytical test instrumentation marketed under the name Luminex¹⁰⁰. This product and the use thereof are covered by one or more of the following US patents: # 6,046,807, # 5,981,180.