

Certificate of Analysis

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

(100 Assay Points)

Catalog # 46-602

Lot # 1384528

Components

Beadlyte® Anti-Erk/MAP Kinase 1/2 Beads, Catalog # 42-602, Lot #1384528. One vial containing 125µl of anti-Erk/MAP Kinase 1/2 rabbit polyclonal IgG conjugated to Luminex® **Bead # 9** at **4000 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 #1384528. 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 Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601).

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

FOR 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 Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) 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. Beadlyte® Cell Signaling Buffer Kits are ordered separately and consist 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: For a detailed protocol on Cell Signaling Detection Procedures please see the COA (select the highest lot number) for the Beadlyte® Cell Signaling Buffer Kit available at:

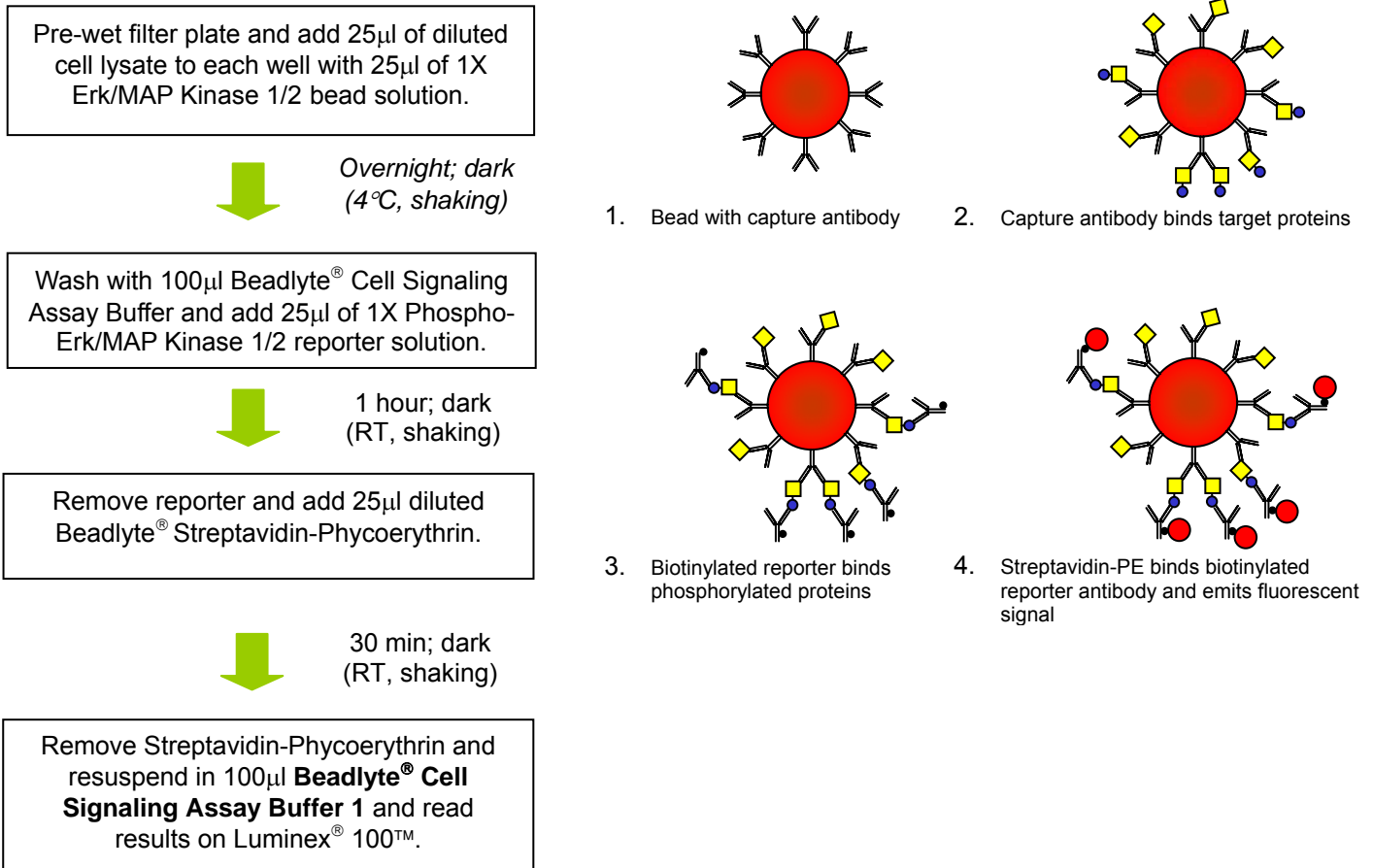
<http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>

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[®] 100™ System
- Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600) or Cell Signaling Universal Buffer Kit (Catalog #48-601)

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 Luminex[®] 100™ 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.



Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer B** (Catalog # 43-019) and Beadlyte® Cell Signaling **Assay Buffer 1** (Catalog # 43-010). Both buffers are included in the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600). For the cell signaling assay and cell lysis protocols refer to the Beadlyte® Cell Signaling Buffer Kit COA (select the highest lot number) at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>.

Multiplex analysis

The recommended lysis and assay buffers multiplexing Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmates™ with other Beadmates™ are Beadlyte® Cell Signaling **Universal Lysis Buffer** (Catalog # 43-040) and Cell Signaling **Universal Assay Buffer** (Catalog # 43-041). Both buffers are included in the Beadlyte® Cell Signaling Universal Buffer Kit (Catalog # 48-601). For the cell signaling assay and cell lysis protocols refer to the Beadlyte® Cell Signaling Universal Buffer Kit COA (select the highest lot number) at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-601>.

For multiplexing Phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) Beadmates™ with other Beadmates™ that are *not* compatible with the Universal Buffer System, select the optimal lysis and assay buffers using the Buffer Selection Table in the Beadlyte® Cell Signaling Buffer Kit COA (Catalog # 48-600). The cell signaling assay and cell lysis protocols are also provided in the Beadlyte® Cell Signaling Buffer Kit COA at: <http://www.upstate.com/browse/productdetail.asp?ProductId=48-600> (select the highest lot number).

Note: Phospho and Total Beadmates should not be multiplexed together.

Representative Data:

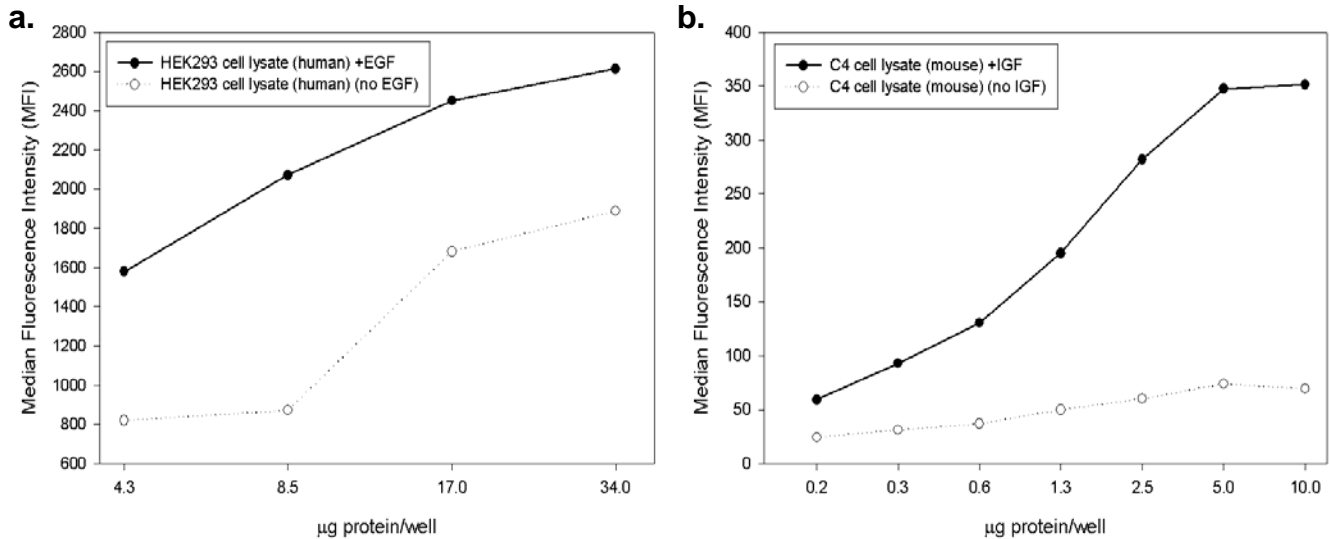
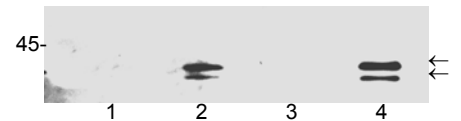


Figure 1. Beadlyte[®] detection of phosphorylated Erk/MAP Kinase 1/2 proteins in HEK293 cell lysate (a) and C4 cell lysate (b). HEK293 cells (a) were grown to confluence and stimulated with (●) or without (○) 50ng/ml of EGF for 10 minutes. C4 cells (b) were grown to confluence and stimulated with (●) 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 Luminex[®] 100™ 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 Corporation, you, the customer, acquire the right under Luminex Corporation'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 Luminex[®] 100™. One or more of the following US patents covers this product and the use thereof: #6,046,807, #5,981,180.