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## Certificate of Analysis

### Total Erk/MAP Kinase 1/2 Beadmates™

(100 Assay Points)

Catalog # 46-609

Lot # 29578

#### Components

**Beadlyte® Anti-Erk/MAP Kinase 1/2 Beads**, Catalog # 42-609, Lot # 29578. 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-Erk/MAP Kinase 1/2, Biotin**, Catalog # 44-609, Lot # 29578. One vial containing **125µl** of anti-Erk/MAP Kinase 1/2 mouse 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 Erk/MAP kinase 1/2, both phosphorylated and non-phosphorylated forms.

**Applications:** Optimal antibody pair for detection of 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**

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#### Total Erk/MAP Kinase 1/2 Beadmate™ Description

**Use:** The Total Erk/MAP Kinase 1/2 Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) to detect the presence of total Erk/MAP Kinase 1/2 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>

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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 $\mu$ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex<sup>®</sup> 100<sup>™</sup> System
- Beadlyte<sup>®</sup> Cell Signaling Buffer Kit (Catalog # 48-600) or Cell Signaling Universal Buffer Kit (Catalog # 48-601)

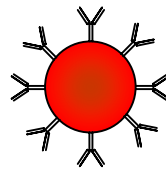
### Detection Protocol Summary

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 overnight with beads coupled to a capture antibody that binds Erk/MAP Kinase 1/2 at a site distinct from the secondary reporter site, capturing both phosphorylated and non-phosphorylated forms of Erk/MAP Kinase 1/2. The beads are washed and mixed with a biotinylated total Erk/MAP Kinase 1/2 specific reporter, followed by streptavidin-phycoerythrin. The amount of total Erk/MAP Kinase 1/2 is then quantified using the Luminex<sup>®</sup> 100<sup>™</sup> System.

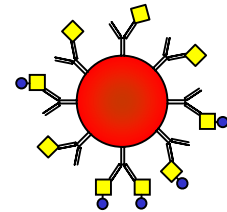
Pre-wet filter plate and add 25 $\mu$ l of diluted cell lysate to each well with 25 $\mu$ l of 1X Total Erk/MAP Kinase 1/2 bead solution.



Overnight; dark  
(4 °C, shaking)



1. Bead with capture antibody

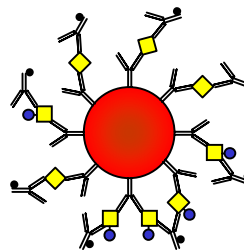


2. Capture antibody binds target proteins

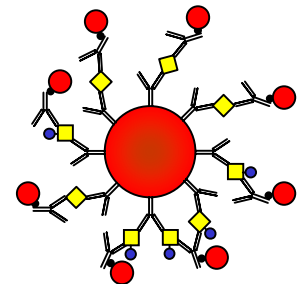
Wash with 100 $\mu$ l Beadlyte<sup>®</sup> Cell Signaling Assay Buffer. Add 25 $\mu$ l of 1X Total Erk/MAP Kinase 1/2 reporter solution.



1 hour; dark  
(RT, shaking)



3. Biotinylated reporter binds proteins



4. Streptavidin-PE binds biotinylated reporter antibody and emits fluorescent signal

Remove reporter and add 25 $\mu$ l diluted Beadlyte<sup>®</sup> Streptavidin-Phycoerythrin.



30 min; dark  
(RT, shaking)

Remove Streptavidin-Phycoerythrin and resuspend in 100 $\mu$ l **Beadlyte<sup>®</sup> Cell Signaling Assay Buffer 1** and read results on Luminex<sup>®</sup> 100<sup>™</sup>.

## Preparations for Assay Protocol

### Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Total Erk/MAP Kinase 1/2 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>.

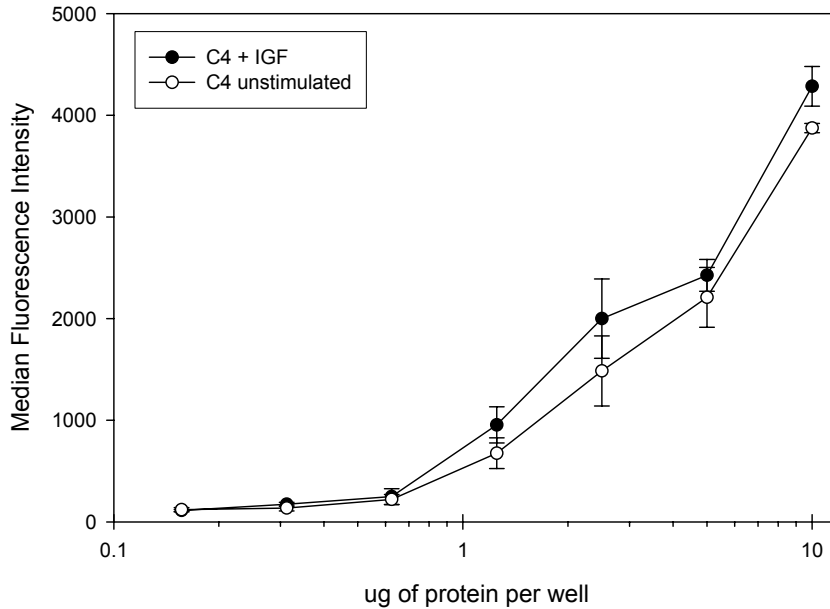
### Multiplex analysis

The recommended lysis and assay buffers multiplexing Total Erk/MAP Kinase 1/2 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>.

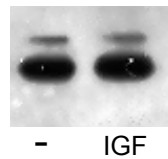
For multiplexing Total Erk/MAP Kinase 1/2 with other Beadmates™ that are *not* compatible with the Universal Buffer System, select the optimal lysis and assay buffers using the Buffer Selection Table in the Beadlyte® Cell Signaling Buffer Kit COA (Catalog # 48-600). The cell signaling assay and cell 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 Total Erk/MAP kinase 1/2 proteins in C4 cell lysate.** C4 cells were grown to confluence and stimulated with (●) or without (○) 50ng/ml of IGF for 15 minutes. Increasing amounts of cell lysate (lysed in Beadlyte® Lysis Buffer B with protease inhibitors) were incubated overnight at 4°C with Beadlyte® Anti-Erk/MAP Kinase 1/2 Capture Beads. The Beads were washed and mixed at room temperature with Beadlyte® Anti-Erk/MAP Kinase 1/2, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the xMAP™ system.



**Figure 2. Western blot detection of Total Erk/MAP kinase 1/2 in C4 cell lysate.** C4 cells were grown to confluence and stimulated with or without 50ng/ml of IGF for 15 minutes. 10µg/well of Unstimulated C4 or IGF stimulated C4 cell lysate (lysed in Beadlyte® Lysis Buffer A with protease inhibitors) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with mouse anti-Erk/MAP Kinase 1/2. Blots were incubated with HRP labeled anti-mouse IgG and visualized via chemiluminescence.

**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.