



cell signaling solutions

Certificate of Analysis

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Beadlyte® Phospho-EGF-Receptor Beadmates™

(100 Assay Points)

Catalog # 46-603

Lot # 22298

Components

Beadlyte® Anti-EGF-R Capture Beads, Catalog # 42-603, Lot # 22298. One vial containing **125µl** of anti-EGF-R mouse monoclonal IgG conjugated to Luminex™ Bead # 34 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® Biotinylated Anti-phosphotyrosine Reporter, Catalog # 44-603, Lot # 22298. One vial containing **125µl** of anti-phosphotyrosine EGF-R mouse monoclonal IgG (**20X**) in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes human phosphorylated EGF-Receptor.

Applications: Optimal antibody pair for detection of phosphorylated EGF-R. 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-EGF-R Beadmate™ Description

Use: The Beadlyte® Phospho-EGF-R Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kit (Catalog # 48-600) to detect the presence of phosphorylated EGF-R in cell lysates using the Luminex¹⁰⁰ LabMAP™ 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 Beadlyte® Phospho-EGF-R Beadmate™ pair CANNOT be multiplexed with the Beadlyte® Total EGF-R Beadmate™ pair (Catalog # 46-606) 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>

Note: It is suggested that 1mM Sodium Orthovanadate be added to the lysis buffer to obtain optimal signal.

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

- Cell lysates or cell extracts harboring protein(s) of interest
- Sodium Orthovanadate
- Vortex mixer
- Plate shaker
- Timer
- Variable volume (5-200 μ l) pipette + tips
- Sonication Bath (Catalog # 40-002)
- Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
- Luminex¹⁰⁰ LabMAP™ System
- Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600)

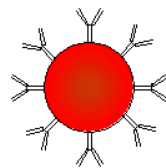
Detection Protocol Summary

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 EGF-R specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho-EGF-R specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-EGF-R 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 EGF-R.

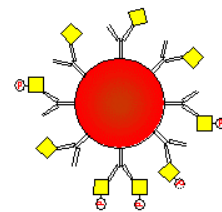
Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X EGF-R bead solution.



*Overnight; dark
(4 °C, shaking)*



1. Bead with capture antibody

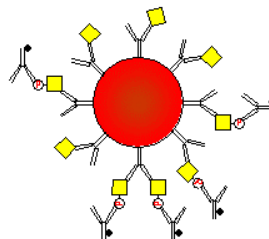


2. Capture antibody binds target proteins

Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l of 1X Phospho-EGF-R reporter solution.



1 hour; dark
(RT, shaking)

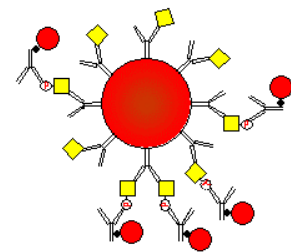


3. Biotinylated reporter binds phosphorylated proteins

Remove reporter and add 25 μ l diluted Beadlyte[®] Streptavidin-Phycoerythrin.



30 min; dark
(RT, shaking)



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

Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex¹⁰⁰.

Recommendations for Protocol

Preparation of lysates

For a single-plex analysis, Beadlyte[®] Cell Signaling **Lysis Buffer A with 1mM Sodium Orthovanadate added** is recommended for lysing cells for Phospho EGF-R 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-EGF-R 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-EGF-R Beads and reporter antibodies

For Phospho-EGF-R 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-EGF-R 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-EGF-R Buffer Selection Chart

Beadmate	Catalog #	Bead #	Lysis buffer	Assay buffer 1 activity (%)	Assay buffer 2 activity (%)	Assay buffer 3 activity (%)
Phospho-EGF-R	46-603	#34	A	100	40-60	60-80
			B	0-20	0	0-20
			C	0	0	0

Representative Data:

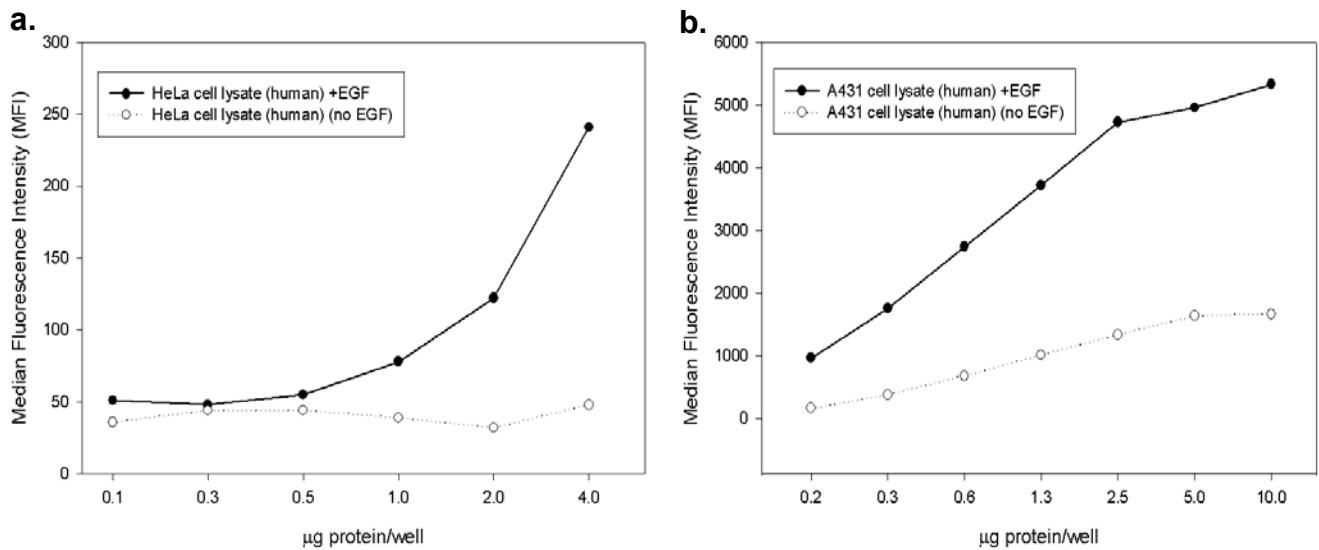
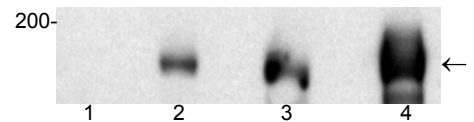


Figure 1. Beadlyte[®] detection of phosphorylated EGF receptor proteins in HeLa (a) and A431 (b) cell lysates. HeLa cells (a) and A431 cells (b) were grown to confluence and stimulated with (●) or without (○) 50ng/ml of EGF for 20 minutes. Increasing amounts of cell lysate (lysed in Beadlyte[®] Cell Signaling Lysis Buffer A with protease inhibitors and 1mM Sodium Orthovanadate) were incubated overnight at 4°C with Beadlyte[®] Anti-EGF Receptor Capture Beads. The Beads were washed and mixed at room temperature with Beadlyte[®] Biotinylated Anti-phosphotyrosine Reporter, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex¹⁰⁰ LabMAP™ system.

Figure 2. Western blot detection of phosphorylated EGF receptor in HeLa and A431 cell lysates. HeLa cells and A431 cells were grown to confluence and stimulated with or without 50ng/ml of EGF for 20 minutes. 10µg/well of unstimulated HeLa (lane 1), stimulated HeLa (lane 2), unstimulated A431 (lane 3) or stimulated A431 (lane 4) cell lysate (lysed in Beadlyte[®] Cell Signaling Lysis Buffer A with protease inhibitors and 1 mM Sodium Orthovanadate) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with mouse monoclonal anti-phospho-tyrosine. Blots were incubated with HRP labeled anti-mouse IgG and visualized via chemiluminescence. Arrow indicates phosphorylated EGF receptor (175 kDa).



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