
Certificate of Analysis

Phospho-I κ B α (Ser32) Beadmates™
(100 Assay Points)
Catalog # 46-643
Lot # 30376

Components

Beadlyte® Anti-I κ B α Beads, Catalog # 42-643, Lot # 30376. One vial containing **125 μ l** of anti-I κ B α IgG conjugated to Luminex™ Bead # 33 at **4000 beads/ μ l (20X)** in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-Phospho-I κ B α (Ser32), Biotin, Catalog # 44-643, Lot # 30376. One vial containing **125 μ l** of anti-phospho-I κ B α IgG (**20X**) in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes human I κ B α phosphorylated on Ser32.

Applications: Optimal antibody pair for detection of I κ B α phosphorylated on Ser32. To be used in conjunction with the 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-I κ B α (Ser32) Beadmate™ Description

Use: The Beadlyte® phospho-I κ B α (Ser32) Beadmate™ pair is used in conjunction with the Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) to detect the presence of phosphorylated I κ B α (Ser32) in cell lysates using the Luminex® xMAP™ 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 a $\text{I}\kappa\text{B } \alpha$ specific capture antibody overnight. The beads are washed and mixed with a biotinylated phospho- $\text{I}\kappa\text{B } \alpha$ (Ser32) specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho- $\text{I}\kappa\text{B } \alpha$ 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 $\text{I}\kappa\text{B } \alpha$.

Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X $\text{I}\kappa\text{B } \alpha$ bead solution.



Overnight; dark
(4°C, shaking)

Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l of 1X phospho- $\text{I}\kappa\text{B } \alpha$ (Ser32) reporter



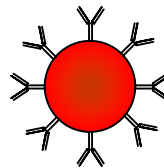
1 hour; dark
(RT, shaking)

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

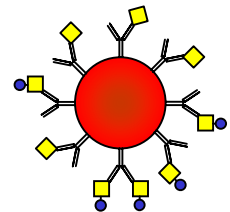


30 min; dark
(RT, shaking)

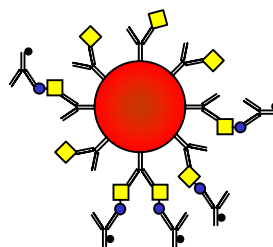
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex[®] 100™ System.



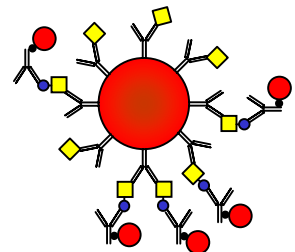
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds phosphorylated proteins



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

Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of phospho-I κ B α (Ser32) 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-I κ B α (Ser32) 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-I κ B α (Ser32) 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:

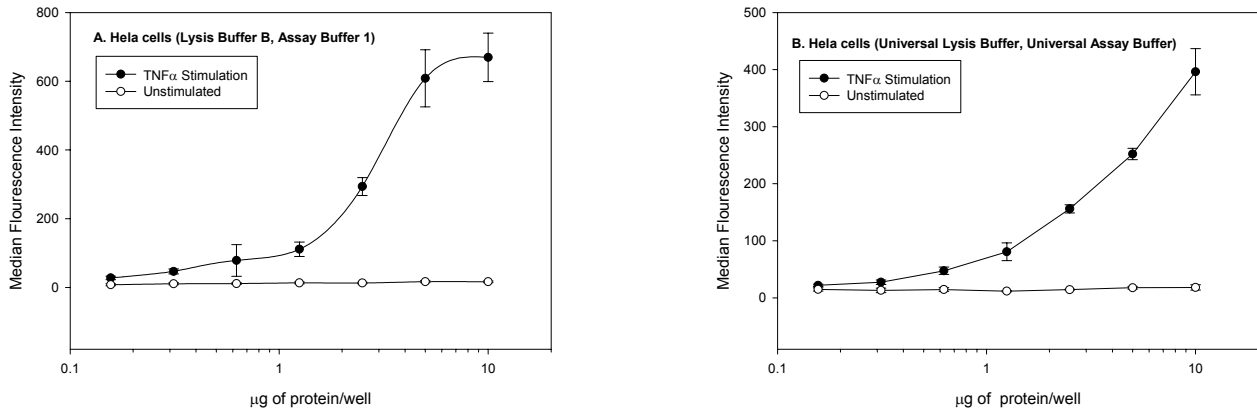


Figure 1. Beadlyte[®] detection of phosphorylated I κ B α (Ser32) in HeLa cell lysate. HeLa cells were grown to 60% confluence, serum starved overnight and stimulated with (●) or without (○) 25ng/ml of TNF α for 7 minutes, and washed with TBS. The cells were then lysed in either Beadlyte[®] Cell Signaling Lysis Buffer B (**Panel A**) or Beadlyte[®] Cell Signaling Universal Lysis Buffer (**Panel B**). The lysates were diluted in Beadlyte[®] Cell Signaling Assay Buffer 1 (**Panel A**) or Beadlyte[®] Cell Signaling Universal Assay Buffer (**Panel B**) and incubated overnight with I κ B α capture beads. The beads were washed and incubated with biotin labeled anti-phospho-I κ B α (Ser32) for 1 hour at 25°C, followed by a 30 minute incubation at 25°C with streptavidin-PE. Beadlyte[®] Cell Signal Amplification Buffer was added to streptavidin-PE (**Panel B**). The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100™ System, and the figure shows the average MFI +/- standard deviation of 3 replicate wells.

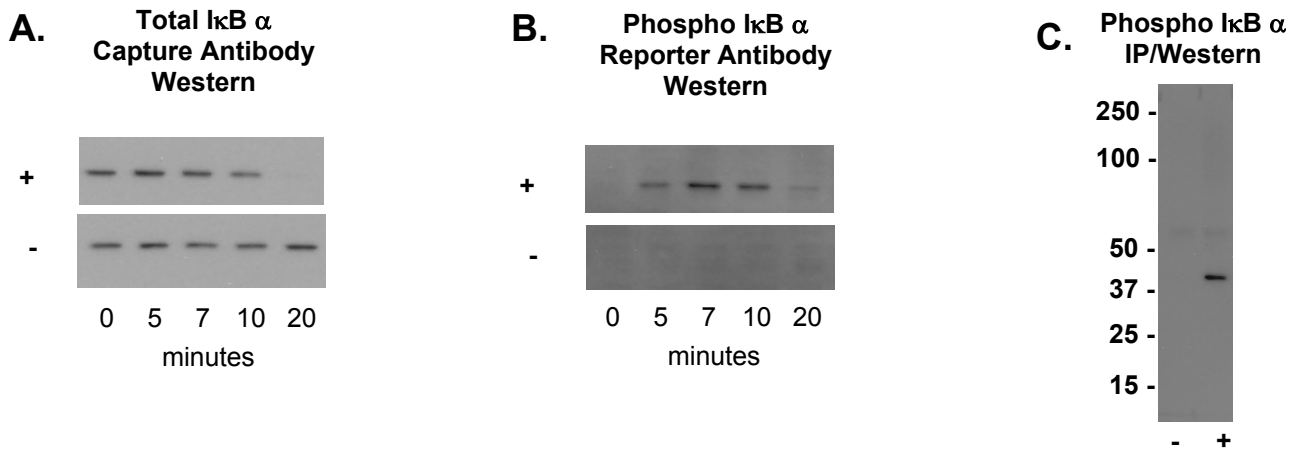


Figure 2. Western blot detection of I κ B α in cell lysates. 5 μ g of lysate from a HeLa cells +/- TNF α time course (prepared as described above) were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-I κ B α capture antibody, (**Panel A.**) or probed with anti-phospho-I κ B α (Ser32) reporter antibody (**Panel B**). I κ B α was immunoprecipitated from HeLa +/- TNF α lysates (7 minute stim., prepared as described above) using anti-I κ B α capture antibody, and probed with anti-phospho-I κ B α (Ser32) reporter antibody (**Panel C.**). All were followed by HRP labeled anti-IgG antibody, 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.