
Certificate of Analysis

Phospho-IRS1 Beadmates™

(100 Assay Points)

Catalog # 46-627

Lot # 26992

Components

Beadlyte® Anti-IRS1 Beads, Catalog # 42-627, Lot # 26992. One vial containing **125µl** of anti-IRS1 IgG conjugated to Luminex® Bead # 36 at **2,000 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-IRS1, Biotin, Catalog # 44-627, Lot # 26992. One vial containing **125µl** of anti-phosphotyrosine IgG **(20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes human, mouse and rat tyrosine-phosphorylated IRS1.

Applications: Optimal antibody pair for detection of tyrosine-phosphorylated IRS1. 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
DO NOT USE FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS
DO NOT USE IN HUMANS OR IN ANIMALS**

Phospho-IRS1 Beadmate™ Description

Use: The Phospho-IRS1 Beadmate™ pair is used in conjunction with Beadlyte® Cell Signaling Buffer Kits (Catalog #s 48-600 or 48-601) to detect the presence of tyrosine-phosphorylated IRS1 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:

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

Detection Protocol Summary

The assay procedure is a 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 an IRS1 specific capture antibody. The beads are washed and mixed with a biotinylated phosphotyrosine specific reporter antibody, followed by streptavidin-phycoerythrin. The amount of tyrosine-phosphorylated IRS1 is then quantified using the Luminex[®] 100[™] System. An unstimulated cell lysate sample containing all other components will give the value for any basal phosphorylated IRS1.

Pre-wet filter plate and add 25 μ l of diluted cell lysate to each well with 25 μ l of 1X **Anti-IRS1 Beads**.



Overnight; dark (4°C, shaking)

Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l of 1X **Anti-Phospho-IRS1, Biotin**.



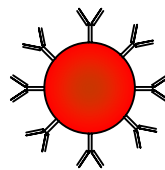
1 hour; dark (RT, shaking)

Wash with 100 μ l Beadlyte[®] Cell Signaling Assay Buffer and add 25 μ l diluted Beadlyte[®] Streptavidin-Phycoerythrin.

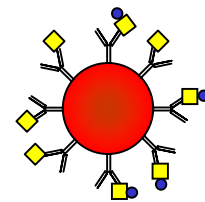


30 min; dark (RT, shaking)

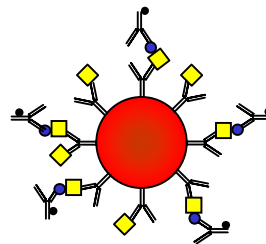
Remove Streptavidin-Phycoerythrin and resuspend in 100 μ l **Beadlyte[®] Cell Signaling Assay Buffer 1** and read results on Luminex[®] 100[™] System.



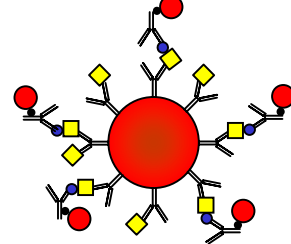
1. Bead with capture antibody



2. Capture antibody binds target proteins



3. Biotinylated reporter binds tyrosine-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-IRS1 Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer B** (Catalog # 43-019) and Beadlyte® Cell Signaling **Assay Buffer 2** (Catalog # 43-011). 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>.

Note: It is recommended that the addition of 1mM sodium orthovanadate be added to 1X Lysis buffer. For a protocol on preparing sodium orthovanadate please see:

<http://www.upstate.com/misc/protocols.asp?prot=activation>

Multiplex analysis

The recommended lysis and assay buffers multiplexing Phospho-IRS1 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-IRS1 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.

Note: The following Beadmates™ use the same anti-phospho-tyrosine biotinylated reporter antibody:

Catalog	Beadmate™
46-603	Phospho-EGF Receptor
46-614	Phospho-PDGFR α
46-615	Phospho-PDGFR β
46-616	Phospho-Lck
46-619	Phospho-cKit

A 1X stock of biotinylated reporter antibody from any one of these Beadmates™ is sufficient for multiplexing two or more of these Beadmates™ together.

Representative Data:

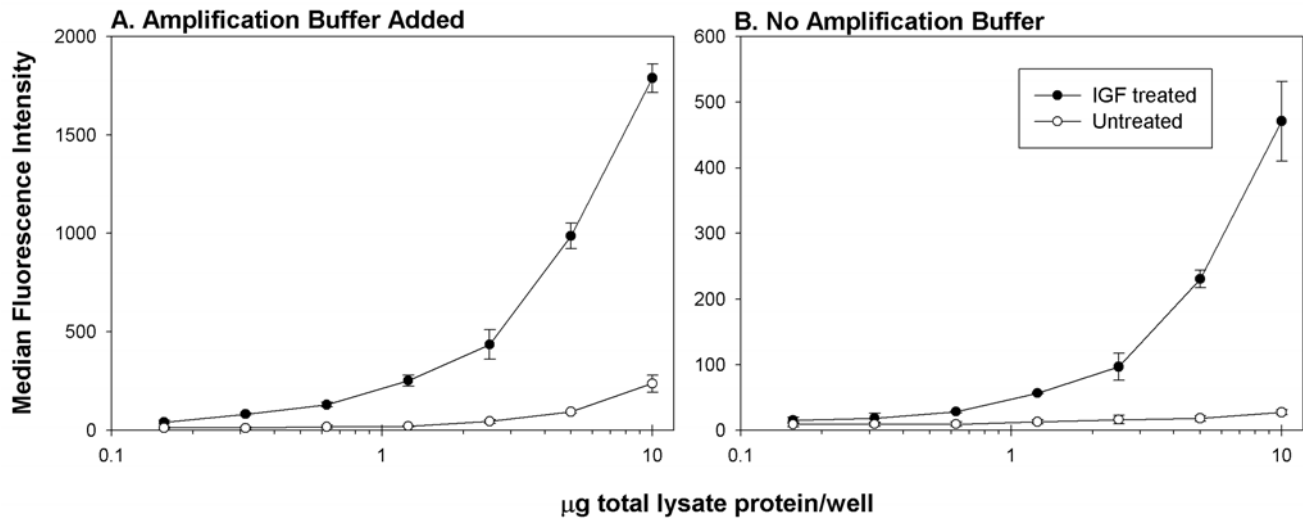


Figure 1. Beadlyte[®] detection of phosphotyrosine-IRS1 in HEK293 cell lysate. HEK293 cells were grown to 85% confluence, serum starved overnight, and treated with (●) or without (○) 50ng/ml IGF-1 for 5 minutes. Cells were lysed in Beadlyte[®] Cell Signaling Lysis Buffer B with protease inhibitors and 5mM Na₃VO₄. Lysates were incubated overnight at 4°C with **Anti-IRS1 Beads**, washed, and incubated for 1 hour with **Anti-Phosphotyrosine-IRS1, Biotin**. The beads were then incubated for 5 minutes with or without Cell Signal Amplification Buffer (Catalog # 43-024), followed by a 30 minute incubation with streptavidin-PE. 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. Immunoprecipitation and Western blot detection of phosphotyrosine-IRS1 in HEK293 cell lysate. HEK293 cells were grown to 85% confluence, serum starved overnight, and treated with (IGF-1) or without (NT) 50ng/ml IGF-1 for 5 minutes. Cells were lysed in Beadlyte[®] Cell Signaling Lysis Buffer C with protease inhibitors and 5mM Na₃VO₄. Protein A-Agarose beads and anti-phosphotyrosine-IRS-1 were incubated together for 1 hour at 4°C and then washed 3X with PBS. Bead-antibody complexes were then incubated with 500µg of total lysate protein for 1 hour at 4°C, and then washed 3X with PBS. The proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-IRS-1. Blots were incubated with an HRP-labeled secondary 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.