
Certificate of Analysis

Total IRS1 Beadmates™

(100 Assay Points)

Catalog # 46-628

Lot # 26993

Components

Beadlyte® Anti-IRS1 Beads, Catalog # 42-628, Lot # 26993. 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-IRS1, Biotin, Catalog # 44-628, Lot # 26993. One vial containing **125µl** of anti-IRS1 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 IRS1.

Applications: Optimal antibody pair for detection of 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**

Total IRS1 Beadmate™ Description

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

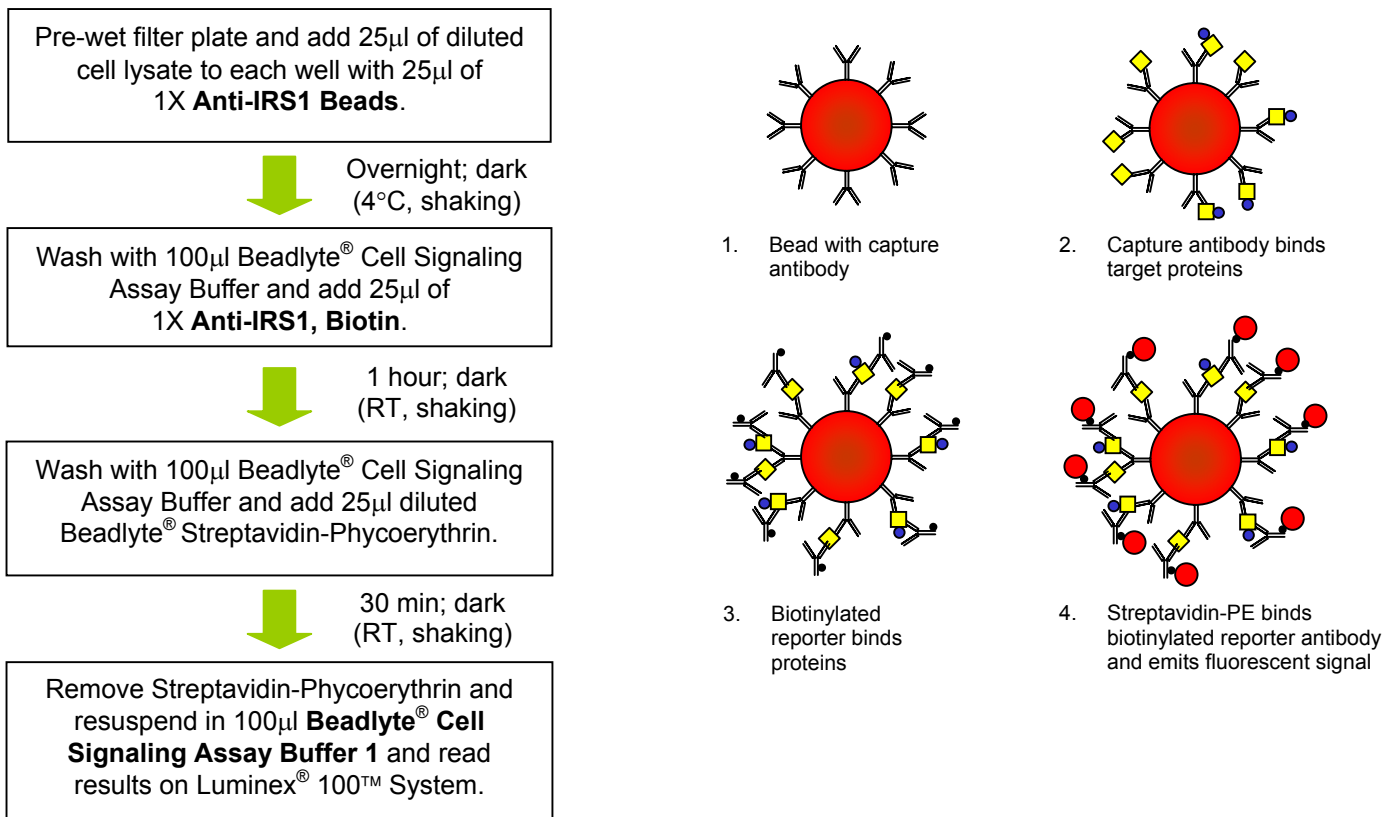
<http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>

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 IRS1 specific reporter antibody, followed by streptavidin-phycoerythrin. The amount of IRS1 is then quantified using the Luminex[®] 100™ System.



Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Total IRS1 Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer B** (Catalog # 43-019) and Beadlyte® Cell Signaling **Assay Buffer 3** (Catalog # 43-012). 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 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 Total 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.

Representative Data:

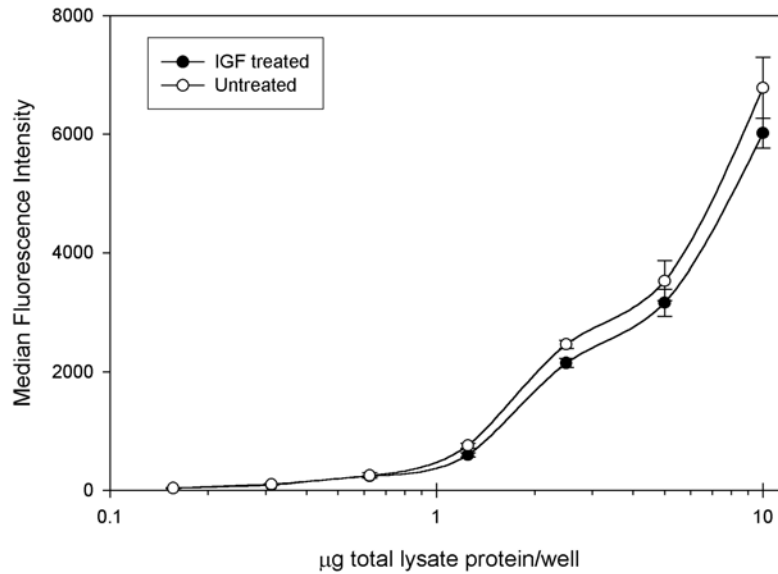
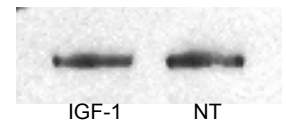


Figure 1. Beadlyte[®] detection of total IRS1 in HEK293 cell lysate. HEK293 cells were grown to 85% confluence, serum starved overnight, and treated with (●) or without (○) 50ng/ml IGF-1 for 5 minutes. Cells were lysed in Beadlyte[®] Cell Signaling Lysis Buffer B with protease inhibitors and 5mM Na₃VO₄. Lysates were incubated overnight at 4°C with **Anti-IRS1 Beads**, washed, and incubated for 1 hour at 25°C with **Anti-IRS1, Biotin**, followed by a 30 minute incubation at 25°C 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. Western blot detection of total 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 B with protease inhibitors and 5mM Na₃VO₄. 10µg of total lysate proteins per well were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-IRS1. 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.