
Certificate of Analysis

Phospho-Lck Beadmates™

(100 Assay Points)

Catalog # 46-616

Lot # 0608038679

Components

Beadlyte® Anti-Lck Beads, Catalog # 42-616, Lot # 0608038679. One vial containing **125µl** of anti-Lck IgG conjugated to Luminex® Bead # 20 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-Lck, Biotin, Catalog # 44-616, Lot # 0608038679. One vial containing **125µl** of anti-phospho-tyrosine IgG (**20X**) in a proprietary formulation of Tris-buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: Recognizes phosphorylated human, rat and mouse Lck.

Applications: Optimal antibody pair for detection of phosphorylated Lck. 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

Phospho-Lck Beadmate™ Description

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

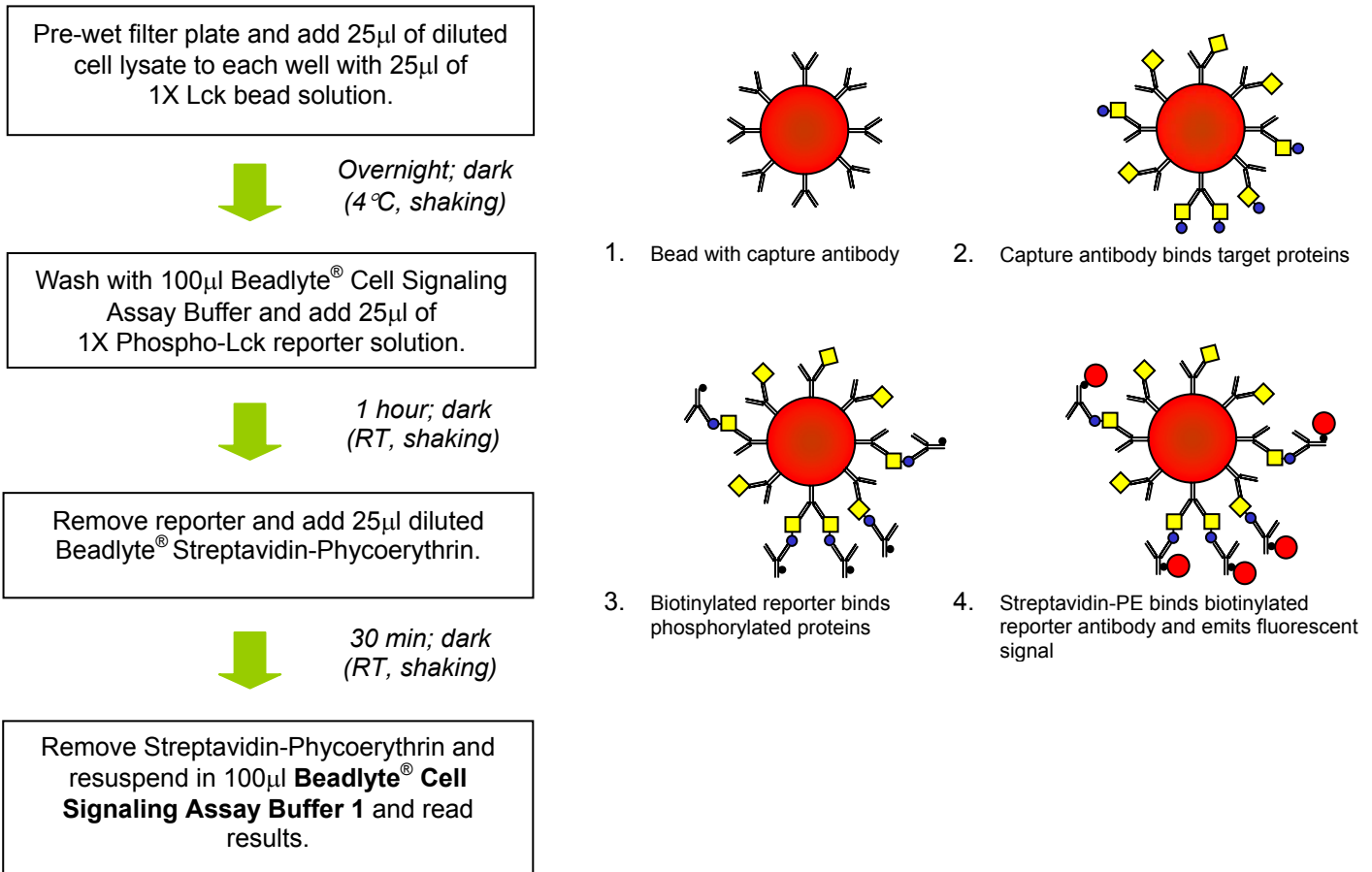
<http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>

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 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 Lck specific antibody. The beads are washed and mixed with a biotinylated phosphotyrosine specific reporter, followed by streptavidin-phycoerythrin. The amount of phospho-Lck is then quantified using the Luminex[®] xMAP™ System. A sample with unstimulated cell lysate and containing all other components will give the value for any basal phosphorylation of Lck.



Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Phospho-Lck Beadmates™ are Beadlyte® Cell Signaling **Lysis Buffer C** (Catalog # 43-020) 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-Lck 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-Lck 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
46-619	Phospho-ckit
46-627	Phospho-IRS1

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:

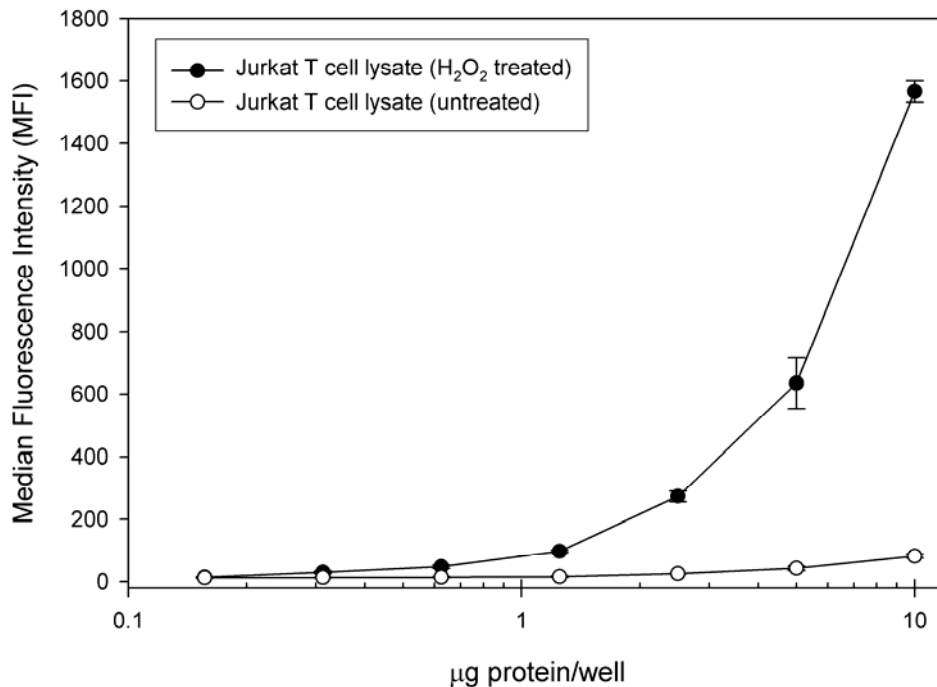
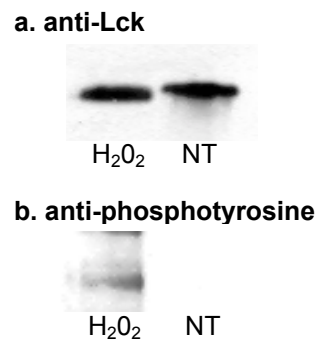


Figure 1. Beadlyte[®] detection of phosphorylated Lck proteins in Jurkat T cell lysate. Jurkat T cells were grown to 90% confluence, serum starved overnight, and stimulated (at 80% confluence) with (●) or without (○) 5mM H₂O₂ for 15 minutes. Increasing amounts of cell lysate (lysed in Beadlyte[®] Cell Signaling Lysis Buffer C with protease inhibitors) were incubated overnight at 4°C with Beadlyte[®] Anti-Lck Beads. The Beads were washed and mixed at room temperature with Beadlyte[®] Anti-Phospho-Lck, Biotin, followed by streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using the Luminex[®] 100™ system.

Figure 2. Western blot detection of total and phospho-Lck in Jurkat T cell lysate. Jurkat T cells were grown, serum starved overnight, and stimulated with (H₂O₂) or without (NT) 5mM H₂O₂ for 15 minutes. Cells were lysed in Beadlyte[®] Cell Signaling Lysis Buffer C. 10µg of total lysate proteins per well were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-Lck (fig. 2a) or anti-phosphotyrosine (fig 2b). 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.