
Certificate of Analysis

Total BAD Beadmates™

(100 Assay Points)

Catalog # 46-695

Lot #

Components

Beadlyte® Anti-BAD Beads, Catalog # 42-695, Lot #. One vial containing **131µl** of anti-BAD rabbit IgG conjugated to Luminex® Bead #59 at **2400 beads/µl (20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Beadlyte® Anti-Total BAD, Biotin, Catalog # 44-695, Lot #. One vial containing **131µl** of biotin conjugated anti-Total BAD mouse IgG **(20X)** in a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Specificity: The assay recognizes epitopes on Total BAD.

Applications: Optimal antibody pair for detection of Total BAD. 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

Total BAD Beadmate™ Description

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

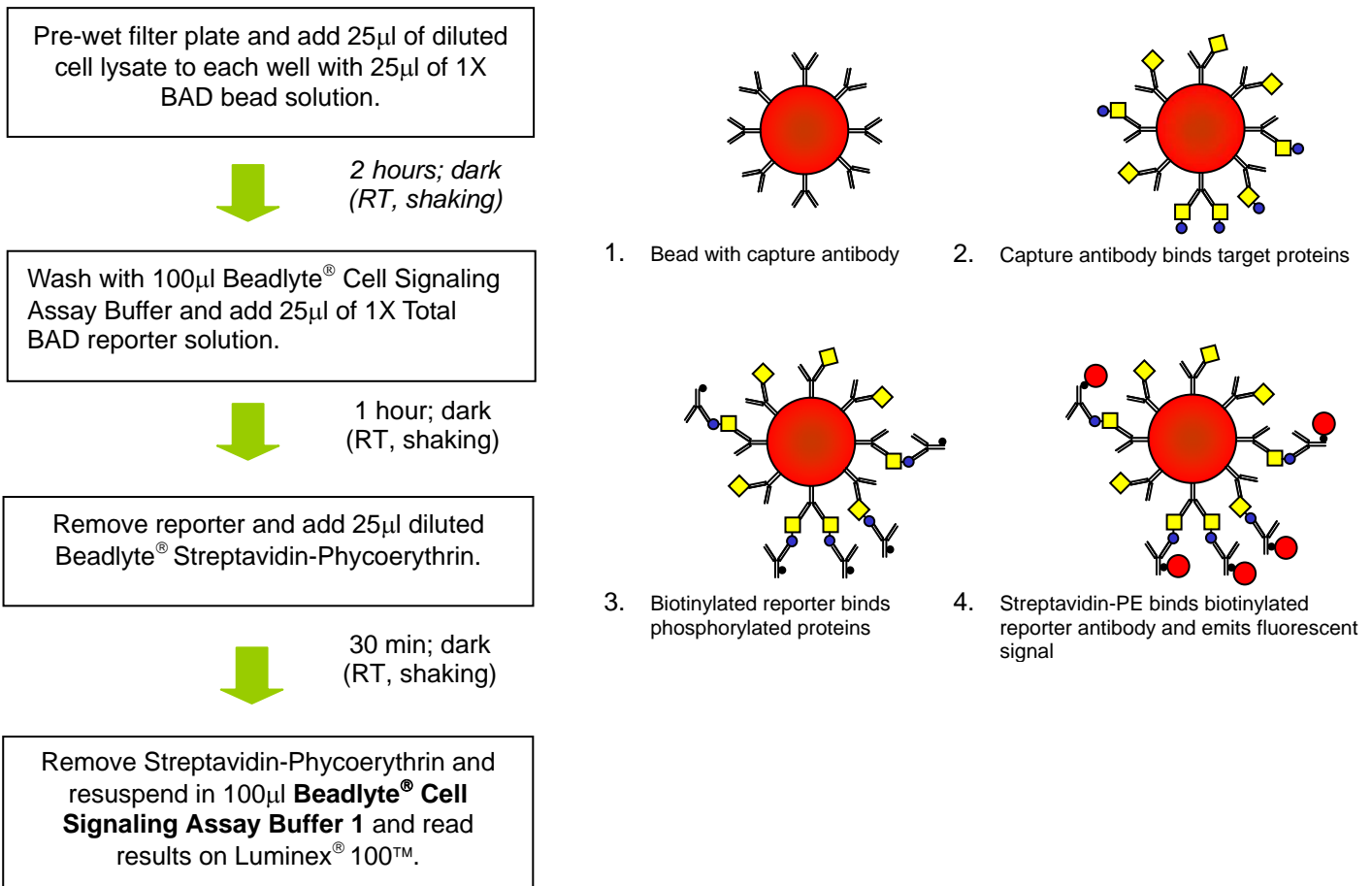
<http://www.upstate.com/browse/productdetail.asp?ProductId=48-600>

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

- 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 BAD specific capture antibody for 2 hours at RT. The beads are washed and mixed with a biotinylated Total BAD specific reporter, followed by streptavidin-phycoerythrin. The amount of total BAD 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 levels of total BAD.



Preparations for Assay Protocol

Single-plex analysis

The recommended lysis and assay buffers for a single-plex analysis of Total BAD Beadmates™ are Beadlyte® Cell Signaling **Universal Lysis Buffer** (Catalog # 43-040) and Beadlyte® Cell Signaling **Assay Buffer 1** (Catalog # 43-010). 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 BAD 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 BAD 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:

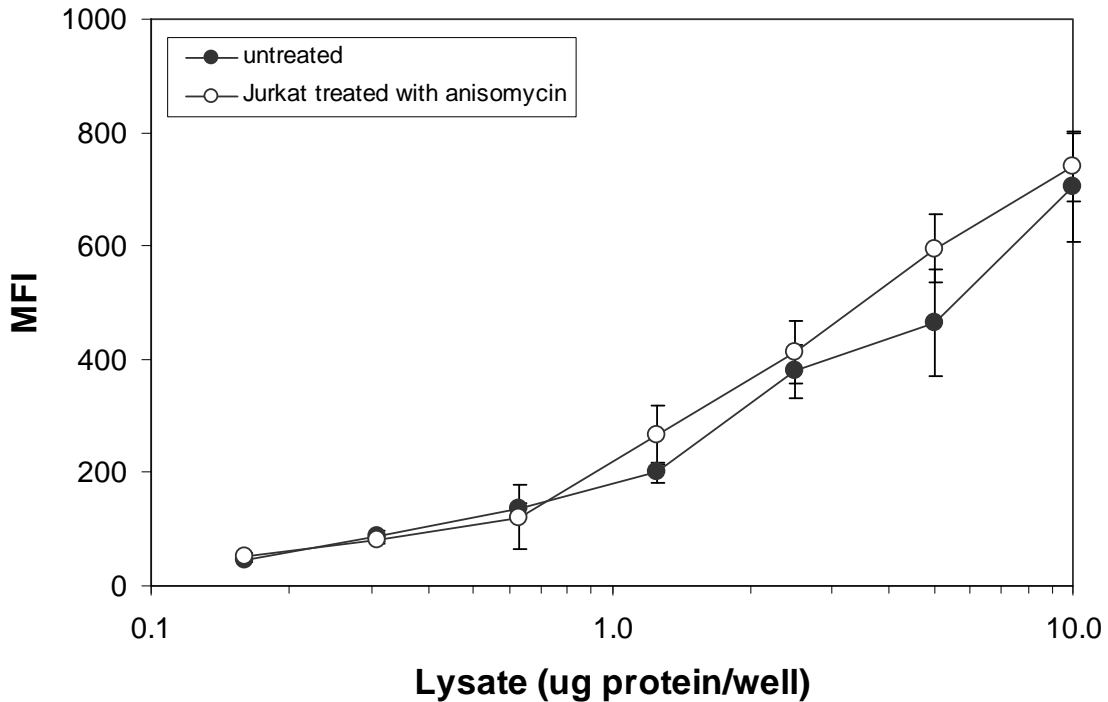


Figure 1. Beadlyte® detection of total BAD in Jurkat cell lysate. Jurkat cells were grown to 1×10^6 cells/ml and stimulated with or without $25 \mu\text{M}$ anisomycin for 4 hours. Increasing amount of cell lysate (lysed in Beadlyte® Universal Lysis Buffer with protease inhibitors) was incubated for 2 hours with anti-BAD capture beads. The beads were washed and mixed at room temperature with biotin labeled anti-total BAD, followed by streptavidin-PE. The Median Fluorescent Intensity (MFI) in triplicate wells was measured using the Luminex® 100™ System. This graph shows average MFI for total BAD as detected with Total BAD Beadmates™.

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.