
Certificate of Analysis

Beadlyte[®] Cell Signal Amplification Buffer

(100 Assay Points)

Catalog # 43-024A

Lot # 0609041300

Formulation: One vial containing **3ml** (100 Assay Points) of a proprietary formulation of Tris buffered salts and animal protein containing 0.05% sodium azide as a preservative.

Use: Add 25 μ l of Cell Signal Amplification Buffer to a Beadlyte[®] Cell Signaling Detection assay prior to addition of streptavidin-phycoerythrin to amplify Median Fluorescence Intensity values.

Sterility: Filtered through a 0.2 μ m membrane.

Storage and Stability: Stable for 1 year at 4°C from date of shipment.

**FOR RESEARCH USE ONLY
DO NOT USE FOR DIAGNOSIS OF DISEASE IN HUMANS OR ANIMALS
DO NOT USE IN HUMANS OR IN ANIMALS**

Product description: Beadlyte[®] Cell Signal Amplification Buffer is used in conjunction with the Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600) or Beadlyte[®] Universal Cell Signaling Buffer Kit (Catalog # 48-601) and Beadlyte[®] Cell Signaling Beadmates[™] for the simultaneous multiplex analysis of cellular events using the Luminex[®] 100[™] system. Addition of the Amplification Buffer prior to the addition of streptavidin-phycoerythrin amplifies the Median Fluorescence Intensity signal and can increase the signal-to-noise ratio.

Other components required but not included are:

- Luminex[®] 100[™] System
 - Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600)
 - Beadlyte[®] Universal Cell Signaling Buffer Kit (Catalog # 48-601)
 - Cell Signaling Beadmates[™] of interest
 - Cell lysates or cell extracts harboring protein(s) of interest
 - Sonication Bath (Catalog # 40-002)
 - Millipore multiscreen vacuum manifold (Catalog # MAVM0960R)
 - Vortex mixer
 - Plate shaker
 - Timer
 - Variable volume (5-200 μ l) pipette + tips
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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.

**Assay Protocol using Cell Signal Amplification Buffer
 Beadlyte[®] Cell Signaling Buffer Kit (Catalog # 48-600)**

Note: changes to Assay Protocol 1 (Standard Assay – see CoA for Catalog # 48-600) are highlighted

1. Pre-wet filter plate with 25µl/well of selected Beadlyte[®] Cell Signaling Assay Buffer. Remove by vacuum filtration by placing the filter plate over a vacuum manifold and gently applying vacuum. Gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
2. Add 25µl of 1X bead suspension to each well.
3. Add 25µl of cell lysate to each well. **NOTE:** Lysates should be diluted at least 1:5 in selected **Assay Buffer** prior to mixing with beads.
4. Incubate overnight on a plate shaker at 4°C protected from light.
5. At the end of the overnight incubation, remove the lysate by vacuum filtration. Gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
6. Add 100µl/well of selected Assay Buffer, remove buffer by vacuum filtration, and gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
7. Add 25µl/well of 1X Beadlyte[®] Biotinylated Reporter.
8. Incubate on a plate shaker for 1 hour at room temperature, protected from light.
9. Remove reporter by vacuum filtration and gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
10. Add 100µl/well of selected Beadlyte[®] Cell Signaling Assay Buffer, remove buffer by vacuum filtration, and gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
11. Add 25µl of Beadlyte[®] diluted (1:25) Beadlyte[®] Streptavidin-Phycoerythrin.
12. Incubate on a plate shaker for 15 minutes at room temperature, protected from light.
13. Add 25µl of Cell Signal Amplification Buffer (Catalog # 43-024).
14. Incubate on a plate shaker for 15 minutes at room temperature, protected from light.
15. Remove Beadlyte[®] Streptavidin-Phycoerythrin by vacuum filtration and gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
16. Resuspend beads in 100µl Beadlyte[®] Cell Signaling **Assay Buffer 1**, place on plate shaker for 1 minute.
***Assay buffers 2 and 3 should not be used to resuspend beads at this point, as they may interfere with the Luminex[®] 100™ instrument analysis.*
17. Analyze using the Luminex[®] 100™ system.

Step	Description
1. Pre-wet filter plate	Add 25µl/well of selected Assay Buffer, vacuum
2. Capture beads	Add 25µl/well of 1X Beads
3. Cell lysate	Add 25µl/well of 1X cell lysate (diluted in Assay Buffer)
4. Overnight incubation	Incubate overnight at 4°C, shaking and protected from light
5. Wash	Remove lysate via vacuum
6. Wash	Wash wells with 100µl Assay Buffer and remove via vacuum
7. Biotin reporter antibody	Add 25µl/well of 1X Biotin-labeled Reporter
8. One hour incubation	Incubate
9. Wash	Remove reporter via vacuum
10. Wash	Wash wells with 100µl Assay Buffer and remove via vacuum
11. Streptavidin-PE	Add 25µl/well of diluted (1:25) Streptavidin-PE
12. 15 minute incubation	Incubate 15 minutes at room temperature, shaking and protected from light
13. Cell Signal Amplification Buffer	Add 25µl/well of Cell Signal Amplification Buffer (Catalog # 43-024) to each well
14. 15 minute incubation	Incubate 15 minutes at room temperature, shaking and protected from light
15. Wash	Remove Streptavidin-PE from wells via vacuum
16. Resuspension in Assay Buffer 1	Add 100µl/well of Assay Buffer 1
17. Analysis	Analyze using the Luminex [®] 100™ system

**Assay Protocol using Cell Signal Amplification Buffer
 Universal Cell Signaling Buffer Kit (Catalog # 48-601)**

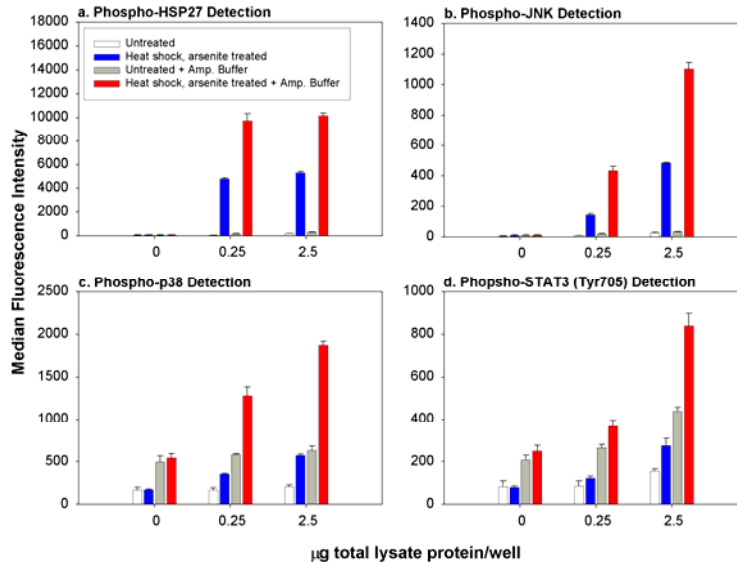
1. Dilute Lysates at least 1:5 in Beadlyte[®] Cell Signaling Universal Assay Buffer. The suggested working range of protein concentration for the assay is 1 to 10 µg of total protein/well (25µl/well at 40 to 400 µg/ml).
2. Pre-wet filter plate with 25µl/well of Beadlyte[®] Cell Signaling Universal Assay Buffer. Remove by vacuum filtration by placing the filter plate over a vacuum manifold and gently applying vacuum. Gently blot the bottom of the filter plate on a paper towel to remove excess liquid.
3. Vortex and sonicate (10 sec) 1X bead suspension, then add 25µl of 1X bead suspension to each well.
4. Add 25µl of diluted cell lysate to each well and incubate overnight¹ at 4°C (or 2 hrs RT) on a plate shaker (600-800 rpm) protected from light.
5. Remove the lysate by vacuum filtration.
6. Add 100µl/well of Beadlyte[®] Cell Signaling Universal Assay Buffer. Remove buffer by vacuum filtration and gently blot the bottom of the filter plate on a paper towel.
7. Wash the beads a second time by repeating step 6.
8. Add 25µl/well of 1X Beadlyte[®] Biotinylated Reporter.
9. Incubate on a plate shaker for 1 hour at room temperature, protected from light.
10. Remove reporter by vacuum filtration and gently blot the bottom of the filter plate on a paper towel.
11. Add 25µl of diluted (1:25) Beadlyte[®] Streptavidin-Phycoerythrin (Strept-PE).
12. Incubate on a plate shaker for 15 minutes at room temperature, protected from light.
13. DO NOT REMOVE Strept-PE. Add 25µl of Beadlyte[®] Cell Signal Amplification Buffer to each well.
14. Incubate on a plate shaker for 15 minutes at room temperature, protected from light.
15. Remove Beadlyte[®] Strept-PE/Amplification buffer by vacuum filtration and gently blot the bottom of the filter plate on a paper towel.
16. Resuspend beads in 100µl of Beadlyte[®] Cell Signaling Universal Assay Buffer.
17. Analyze using the Luminex[®] 100™ system.

Step	Description
1. Prepare cell lysate samples	Dilute cell lysates in Beadlyte [®] Cell Signaling Universal Assay Buffer
2. Pre-wet filter plate	Add 25µl/well of Beadlyte [®] Cell Signaling Universal Assay Buffer, vacuum
3. Capture beads	Add 25µl/well of 1X Beads
4. Cell lysate/incubation	Add 25µl/well of 1X cell lysate and incubate 2 hrs RT or overnight ¹ at 4°C, shaking and protected from light
5. Remove lysate	Remove lysate via vacuum
6. Wash	Wash wells with 100µl Beadlyte [®] Cell Signaling Universal Assay Buffer
7. Second wash	Repeat step 6 a second time
8. Biotin reporter antibody	Add 25µl/well of 1X Biotin-labeled Reporter
9. One hour incubation	Incubate 1 hr RT
10. Remove Reporter	Remove reporter via vacuum
11. Streptavidin-PE	Add 25µl/well of Streptavidin-PE (diluted 1:25)
12. 15 minute incubation	Incubate 15 minutes at room temperature, shaking and protected from light
13. Amplification buffer	DO NOT REMOVE STREPTAVIDIN-PE. Add 25µl of Cell Signal Amplification Buffer to each well containing 25µl of Streptavidin-PE
14. 15 min incubation	Incubate 15 min at room temperature, shaking and protected from light
15. Remove streptavidin-PE/Amplification buffer	Remove Streptavidin-PE/Amplification buffer from wells via vacuum
16. Resuspension in Beadlyte [®] Cell Signaling Universal Assay Buffer	Add 100µl/well of Beadlyte [®] Cell Signaling Universal Assay Buffer
17. Analysis	Analyze using the Luminex [®] 100™ system

¹ Beadmates™ can be incubated with cell lysate for 2 hrs at room temperature. However, overnight incubations provide better sensitivity.

Representative Data:

Multiplex analysis of phospho-HSP27, phospho-JNK, phospho-p38, and phospho-STAT3 (Tyr705) in HeLa cells



Phospho-HSP27 (Cat. # 46-607)	0 µg		0.25 µg		2.5 µg	
	Avg. MFI +/- SD	Avg. MFI +/- SD	Signal:Noise	Avg. MFI +/- SD	Signal:Noise	
Lysate						
Untreated	46 +/- 6	45 +/- 5	1.0	175 +/- 9	3.8	
Heat shock, arsenite treated	54 +/- 14	4779 +/- 99	88.5	5312 +/- 111	98.4	
Untreated + Amp. Buffer	47 +/- 6	166 +/- 16	3.5	285 +/- 29	6.1	
Heat shock, arsenite treated + Amp. Buffer	57 +/- 2	9738 +/- 583	170.8	10174 +/- 210	178.5	

Phospho-JNK (Cat. # 46-613)	0 µg		0.25 µg		2.5 µg	
	Avg. MFI +/- SD	Avg. MFI +/- SD	Signal:Noise	Avg. MFI +/- SD	Signal:Noise	
Lysate						
Untreated	5 +/- 1	9 +/- 1	1.8	26 +/- 5	5.2	
Heat shock, arsenite treated	9 +/- 4	144 +/- 9	16.0	486 +/- 1	54.0	
Untreated + Amp. Buffer	10 +/- 4	20 +/- 2	2.0	34 +/- 2	3.4	
Heat shock, arsenite treated + Amp. Buffer	11 +/- 4	434 +/- 29	39.5	1103 +/- 41	100.3	

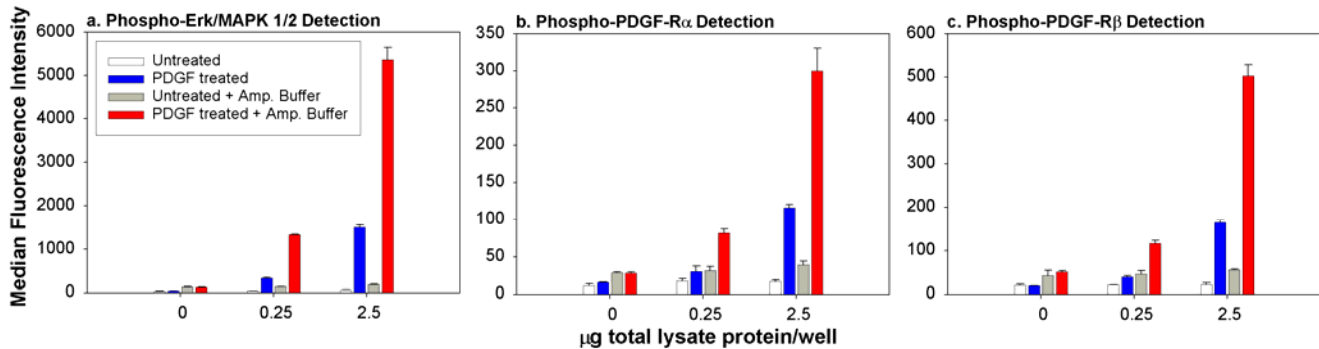
Phospho-p38 (Cat. # 46-610)	0 µg		0.25 µg		2.5 µg	
	Avg. MFI +/- SD	Avg. MFI +/- SD	Signal:Noise	Avg. MFI +/- SD	Signal:Noise	
Lysate						
Untreated	167 +/- 34	167 +/- 30	1.0	208 +/- 20	1.2	
Heat shock, arsenite treated	172 +/- 9	353 +/- 9	2.1	572 +/- 18	3.3	
Untreated + Amp. Buffer	497 +/- 70	582 +/- 9	1.2	632 +/- 52	1.1	
Heat shock, arsenite treated + Amp. Buffer	543 +/- 47	1281 +/- 102	2.4	1869 +/- 51	3.4	

Phospho-STAT3 (Tyr705) (Cat. # 46-623)	0 µg		0.25 µg		2.5 µg	
	Avg. MFI +/- SD	Avg. MFI +/- SD	Signal:Noise	Avg. MFI +/- SD	Signal:Noise	
Lysate						
Untreated	84 +/- 27	87 +/- 23	1.0	156 +/- 11	1.9	
Heat shock, arsenite treated	80 +/- 5	123 +/- 9	1.5	275 +/- 35	3.4	
Untreated + Amp. Buffer	208 +/- 23	266 +/- 16	1.3	439 +/- 20	2.1	
Heat shock, arsenite treated + Amp. Buffer	249 +/- 27	368 +/- 28	1.5	838 +/- 60	3.4	

Signal:Noise is defined as Avg. MFI of three replicate wells containing lysate divided by Avg. MFI of three replicate wells containing 0µg lysate.

Beadlyte® Cell Signal Amplification Buffer increases the MFI signal and can increase the signal-to-noise ratio in multiplex analysis of intracellular signaling pathways in HeLa cells. HeLa cells were grown to 80% confluence, heat shocked at 42°C for 30 minutes, incubated 16 hours at 37°C, and then treated with 400µM arsenite for 30 minutes. Untreated HeLa cells were kept at 37°C for 30 minutes and incubated 16 hours at 37°C without arsenite treatment. Lysates were prepared by lysing cells in Cell Signaling Lysis Buffer B containing 5mM Na₃VO₄ and protease inhibitors. Multiplex experiments were performed by incubating capture beads and cell lysate together overnight at 4°C. The beads were washed and incubated with biotinylated reporters for 1 hour at 25°C. Next, the beads were washed and incubated 5 minutes with or without Cell Signal Amplification Buffer followed by a 30 minute incubation with streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using a Luminex® 100™ system. The figures show the average MFI +/- standard deviation of three replicate wells.

Multiplex analysis of phospho-Erk/MAPK 1/2, phospho-PDGF-R α , and phospho-PDGF-R β in NIH3T3 cells



Phospho-Erk/MAPK 1/2 (Cat. # 46-602)	0 μ g		0.25 μ g		2.5 μ g	
Lysate	avg. MFI +/- SD	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD
Untreated	40 +/- 1	47 +/- 1	1.2	64 +/- 3	1.6	
PDGF treated	42 +/- 5	332 +/- 14	7.9	1505 +/- 60	35.8	
Untreated + Amp. Buffer	126 +/- 14	142 +/- 3	1.1	190 +/- 9	1.5	
PDGF treated + Amp. Buffer	123 +/- 11	1335 +/- 8	10.9	5367 +/- 282	43.6	

Phospho-PDGF-R α (Cat. # 46-614)	0 μ g		0.25 μ g		2.5 μ g	
Lysate	avg. MFI +/- SD	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD
Untreated	12 +/- 3	18 +/- 3	1.5	17 +/- 2	1.4	
PDGF treated	16 +/- 1	31 +/- 7	1.9	116 +/- 5	7.3	
Untreated + Amp. Buffer	29 +/- 2	31 +/- 6	1.1	39 +/- 5	1.3	
PDGF treated + Amp. Buffer	28 +/- 2	82 +/- 6	2.9	300 +/- 31	10.7	

Phospho-PDGF-R β (Cat. # 46-615)	0 μ g		0.25 μ g		2.5 μ g	
Lysate	avg. MFI +/- SD	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD	Signal:Noise	avg. MFI +/- SD
Untreated	21 +/- 3	21 +/- 1	1.0	22 +/- 4	1.0	
PDGF treated	19 +/- 1	39 +/- 4	2.1	165 +/- 5	8.7	
Untreated + Amp. Buffer	43 +/- 12	47 +/- 8	1.1	55 +/- 3	1.2	
PDGF treated + Amp. Buffer	51 +/- 3	117 +/- 7	2.3	502 +/- 26	9.8	

Signal:Noise is defined as Avg. MFI of three replicate wells containing lysate divided by Avg. MFI of three replicate wells containing 0 μ g lysate.

Beadlyte[®] Cell Signal Amplification Buffer increases the MFI signal and can increase the signal-to-noise ratio in multiplex analysis of intracellular signaling pathways in 3T3 cells. NIH3T3 cells were grown to 80% confluence, serum starved overnight, and treated with or without 50ng/ml of PDGF-AB for 5 minutes. Lysates were prepared by lysing cells in Cell Signaling Lysis Buffer B containing 5mM Na₃VO₄ and protease inhibitors. Multiplex experiments were performed by incubating capture beads and cell lysate together overnight at 4°C. The beads were washed and incubated with biotinylated reporters for 1 hour at 25°C. Next, the beads were washed and incubated 5 minutes with or without Cell Signal Amplification Buffer followed by a 30 minute incubation with streptavidin-PE. The Median Fluorescence Intensity (MFI) was measured using a Luminex[®] 100™ system. The figures show the average MFI +/- standard deviation of three replicate wells.