

Bio-Plex™ Phosphoprotein and Total Target Assays



Phosphoprotein Detection
Quick Guide

BIO-RAD

Calculations

| | Phosphoprotein Assay | Total Target Assay |
|---|---|--|
| Total number of wells (includes 25% excess) | _____ wells x 1.25% = _____ wells a | _____ wells x 1.25% = _____ wells a |
| Coupled Beads | | |
| Volume of coupled beads (50x) | 1 µl/well x _____ wells = _____ µl a b | 1 µl/well x _____ wells = _____ µl a b |
| Volume of coupled beads (1x) | _____ wells x 50 µl/well = _____ µl a c | _____ wells x 50 µl/well = _____ µl a c |
| Volume of wash buffer | _____ µl - _____ µl = _____ µl c b | _____ µl - _____ µl = _____ µl c b |
| Detection Antibody | | |
| Volume of detection antibodies (25x) | 1 µl/well x _____ wells = _____ µl a b | 1 µl/well x _____ wells = _____ µl a b |
| Volume of streptavidin-PE (100x) | _____ wells x 25 µl/well = _____ µl a c | _____ wells x 25 µl/well = _____ µl a c |
| Volume of detection antibody diluent | _____ µl - _____ µl = _____ µl c b | _____ µl - _____ µl = _____ µl c b |
| Streptavidin-PE | | |
| | _____ wells x 0.5 µl/well = _____ µl a b | |
| Volume of streptavidin-PE (1x) | _____ wells x 50 µl/well = _____ µl a c | |
| Volume of wash buffer | _____ µl - _____ µl = _____ µl c b | |

Legend

Wash = Add 100 µl of the wash buffer to each well. Place the filter plate on a calibrated vacuum apparatus and remove the buffer by vacuum filtration. Blot the bottom of the filter plate with a clean paper towel. Repeat as specified.

Rinse = Add 100 µl of the resuspension buffer to each well. Place the filter plate on a calibrated vacuum apparatus and remove buffer by vacuum filtration. Blot the bottom of the filter plate with a clean paper towel. Repeat as specified.

Incubate = Cover the filter plate with a new sheet of sealing tape. Place the filter plate on a microplate shaker and then cover with aluminum foil. Shake the filter plate at room temperature at 1,100 rpm for 30 sec, then at 300 rpm for the specified incubation time.

Vacuum filter = Place the filter plate on a calibrated vacuum apparatus and remove the buffer by vacuum filtration. Blot the bottom of the filter plate with a clean paper towel.

Acquire data = Remove the sealing tape and acquire data on the Bio-Plex suspension array system (or Luminex system) according to the recommendations stated in the Bio-Plex phosphoprotein detection instruction manual.

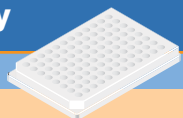
Preparation

Assay

Day 1

Dilute coupled beads to 1x with wash buffer

Prepare lysates in advance as specified in the Bio-Plex cell lysis kit



Wash (1x)

50 μ l/well



Vacuum filter
Wash (2x)

50 μ l/well



Day 2

Dilute detection antibodies to 1x with detection antibody diluent

Dilute streptavidin-PE to 1x with wash buffer

Add resuspension buffer



Incubate 15–18 hr

Vacuum filter
Wash (3x)

25 μ l/well



Incubate (30 min)
Vacuum filter
Wash (3x)

50 μ l/well

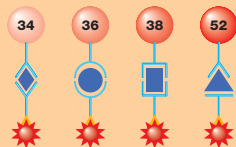


Incubate (10 min)
Vacuum filter
Rinse (3x)

125 μ l/well



Incubate (30 sec)
Acquire data



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What You Need for Phosphoprotein Detection

1



Bio-Plex phosphoprotein assays and Bio-Plex total target assays — includes coupled beads, detection antibodies, and lysates to test for specific proteins of interest in lysate samples.

2



Bio-Plex cell lysis kit — includes cell lysis buffer, cell wash buffer, factor 1 and factor 2; essential for optimal lysis of your cell culture or tissue samples.

3



Bio-Plex phosphoprotein detection reagent kit — includes assay buffer, wash buffer, antibody diluent, resuspension buffer, streptavidin-PE, 96-well filter plate, sealing tape, and instructions required to prepare assays and acquire data.

Note: Phosphoprotein and total target assays require the same reagent kit and cell lysis kit.

For more information on phosphoprotein detection, request bulletins 2903, 2905, 2981, 3033, and 3034.

Available Assays

For a current list of Bio-Plex phosphoprotein and total target assays, contact your local Bio-Rad sales representative or visit us on the Web at www.bio-rad.com/bio-plex/



Bio-Plex phosphoprotein and total target assays feature CST antibodies.