

**Bio-Plex™** Phosphoprotein and Total Target Assays



Phosphoprotein Detection  
**Quick Guide**

**BIO-RAD**

## Calculations

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

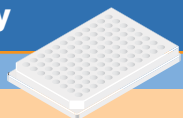
# Preparation

# 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  $\mu$ l/well



Vacuum filter  
Wash (2x)

50  $\mu$ 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  $\mu$ l/well



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

50  $\mu$ l/well

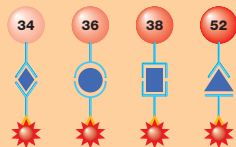


Incubate (10 min)  
Vacuum filter  
Rinse (3x)

125  $\mu$ 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/](http://www.bio-rad.com/bio-plex/)



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