Bio-Plex Pro Assay 384-Well Protocol

Protocol

Introduction

The current Bio-Plex Pro protocols are designed for multiplex assays in a 96-well plate format. Here we detail a recommended protocol for a 384-well plate format that allows for the analysis of more samples and uses smaller sample volumes. The number of kits required may be adjusted for partial plate use. Follow the kit instructions with the following exceptions and recommendations.

Required Materials

- To run a full 384-well plate, use either 6 kits containing 20x beads or 7 kits containing 10x beads. Ensure that all kits have the same batch/lot number. If you are unsure of the bead concentration, please contact your local Bio-Rad support team at bio-rad.com/support
- 384-well handheld magnetic plate washer (Flick and Blot Magnetic Separation Plate for 384 Well Flat, Round, V, or Pyramid Bottom Microplates [catalog #VP 771HH-Q, V&P Scientific, Inc.])

Standard Curve

- For use with a 384-well plate, generate a 10-point standard curve. Prepare the standard curve according to the directions in the kit instruction manual. Continue the serial dilutions until you have 10 vials
- Standards should be run in quadruplicate

Bead Preparation

- Vortex coupled beads for 30 sec
- Pool 4 tubes of 20x or 4 tubes of 10x stock beads before diluting
- Dilute to 4x as shown in Table 1

Table 1. Dilution for beads.

Bead Concentration	Beads, µl	Assay Buffer Diluent, µl	Final Volume (4x), µl
10x	2,000	3,000	5,000
20x	1,000	4,000	5,000

Note: To obtain the same final concentration of beads used in the 96-well Bio-Plex Pro Assay format, beads need to be diluted to 4x.

Standards, Samples, and Controls

- Standards, samples, and controls should be run in quadruplicate
- Vortex 4x diluted beads and add 12.5 µl/well
- Wash the plate 2 times with 25 µl per well of 1x wash buffer using the 384-well handheld magnetic plate washer

  Tip: When using a magnetic plate holder, make sure the plate is clipped in tightly so it doesn't fall out when flipped over.

  Tip: When discarding the wash buffer, firmly grasp the magnetic plate holder in one hand and do a forceful throw down motion into a waste container to reduce residual fluid and bubbling at the top of the wells. Do two to three more rapid flicks keeping the holder inverted and finally patting it dry on paper towels.
- Vortex samples, standards, blank, and controls, and add 12.5 µl/well. Cover with a foil seal and incubate according to the directions in the kit instruction manual

Detection Antibody

1. Pool six tubes of 20x or seven tubes of 10x detection antibody and dilute to 4x as shown in Table 2.

Table 2. Dilution for detection antibody.

Detection Antibody Concentration	Detection Antibody, µl	Detection Antibody Diluent, µl	Final Volume (4x), µl
10x	2,000	3,000	5,000
20x	1,000	4,000	5,000

2. Wash the plate three times with 25 µl per well of 1x wash buffer using the 384-well handheld magnetic plate washer.

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3. Vortex diluted 4x detection antibody and add 12.5 µl/well.
4. Cover the plate with a foil seal and incubate according to the directions in the kit instruction manual.

Streptavidin-Phycoerythrin

1. Prepare 4x streptavidin-phycoerythrin (SA-PE) 5 min before the detection antibody incubation ends as shown in Table 3.

Table 3. Dilution of SA-PE.

Number of Wells	100x SA-PE, µl	Assay Buffer, µl	Final Volume (4x), µl
384	200	4,800	5,000

2. Wash the plate three times with 25 µl per well of 1x wash buffer using the 384-well handheld magnetic plate washer.
3. Vortex diluted 4x SA-PE and add 12.5 µl/well.
4. Cover the plate with a foil seal and incubate according to the directions in the kit instruction manual.
5. Wash the plate three times with 25 µl per well of 1x wash buffer using the 384-well handheld magnetic plate washer.
6. Resuspend the beads in 70 µl of assay buffer. Cover and incubate according to the directions in the kit instruction manual.
7. Read the plate in a Bio-Plex 3D Suspension Array System.

Visit bio-rad.com/bio-plex for more information.

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