# Bio-Plex Pro™ Assays

## Quick Guide 3

**For Use With:**

| Product Family | Instruction Manual # |
|---|---|
| Bio-Plex Pro cell signaling assays | 10024929 |

This guide describes how to prepare and run a full 1 x 96-well flat bottom plate using a magnetic assay workflow. For more information on a given step, refer to the corresponding section of the complete instruction manual. New users can download the manual, which includes detailed instructions and a list of kit components, at **www.bio-rad.com/bio-plex**.

**IMPORTANT!** Pay close attention to **vortexing**, **shaking**, and **incubation instructions**. Deviation from the protocol may result in low assay signal and assay variability. **Do Not** mix phosphoprotein assays with corresponding total target assays (e.g., phospho-Akt and total Akt). Use only the reagents supplied with cell signaling assay kits.

### Sample Preparation

1. Prepare lysates from cell culture or tissue samples. To remove insoluble debris from lysates (≤**2 ml**), centrifuge at **15,000 x g** for **10 min** at 4˚C in a benchtop microfuge. (**Section 1** of the complete instruction manual)
2. Measure protein concentration using the Bio-Rad® *DC*™ protein assay.
3. Adjust protein concentration to **20–200 µg/ml** in Bio-Plex® cell lysis buffer containing cell lysis factor QG and PMSF. For samples with unknown target expression or phosphorylation levels, start with **200 µg/ml** protein.
4. If necessary, store lysates at –70˚C.
5. Thaw frozen lysates and keep on ice. Centrifuge again as above and bring to room temperature (RT) immediately before use.

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## Assay — Day 1

**Note:** Make sure all assay components are at RT before use.

1. Plan the plate layout. (**Section 2** of the complete instruction manual)
2. Prime wash station or prepare handheld magnetic washer. (**Section 3**)
3. Reconstitute Bio-Rad cell lysates in **250 µl** of di$H_2O$ per vial. **Vortex** for **5 sec** and incubate at RT for **20 min**. Transfer the cell lysate to a centrifuge tube and centrifuge at **15,000 x g** for **10 min** at 4°C. Protein concentration is now **200 µg/ml**. (**Section 4**)
4. **Vortex** coupled beads for **30 sec** and dilute to 1x in cell signaling wash buffer as shown below. Protect from light. (**Section 4**)

| # of Wells | 20x Beads, µl | Wash Buffer, µl | Total Volume, µl |
|---|---|---|---|
| 96 | 288 | 5,472 | 5,760 |

5. **Vortex** the diluted (1x) beads. Add **50 µl** to each well of the assay plate.
6. Wash the plate two times with **200 µl** wash buffer.
7. Gather samples, Bio-Rad cell lysates, and blank (detection antibody diluent alone). Add **50 µl** to each well. Recommended total amount of protein to add is **1–10 µg** per well.
8. Cover and incubate in the dark overnight (**15–18 hrs**) at RT with shaking.

**Note:** To resuspend the beads, shake vigorously at **900–1,100 rpm** for **30 sec**. Slowly ramp up to speed to avoid splashing. Then turn down to **300–450 rpm** for the specified incubation time.

## Assay — Day 2 (Section 4 unless otherwise noted)

1. Start up/warm up the Bio-Plex system (may take up to **30 min**). (**Section 5**)
   Meanwhile, bring buffers and diluents to RT.

2. Prime wash station or prepare handheld magnetic washer. (**Section 3**)
3. Calibrate the Bio-Plex system by following the prompts within Bio-Plex Manager™ software. (**Section 5**)
4. With **10 min** left in the overnight incubation, **vortex** detection antibodies for **5 sec** and quick-spin to collect liquid. Dilute to 1x in detection antibody diluent as shown below.

| # of Wells | 20x Detection Ab, µl | Detection Ab Diluent, µl | Total Volume, µl |
|---|---|---|---|
| 96 | 150 | 2,850 | 3,000 |

5. Wash the plate three times with **200 µl** wash buffer.
6. **Vortex** the diluted (1x) detection antibodies. Add **25 µl** to each well.
7. Cover and incubate in the dark for **30 min** at RT. Shake at **900–1,100 rpm** for **30 sec** and then turn down to **300–450 rpm** for the specified incubation time.

   Meanwhile, prepare the Bio-Plex Manager software protocol. (**Section 5**)
8. With **10 min** left, **vortex** streptavidin-PE (SA-PE) for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown below and protect from light.

| # of Wells | 100x SA-PE, µl | Detection Ab Diluent, µl | Total Volume, µl |
|---|---|---|---|
| 96 | 60 | 5,940 | 6,000 |

9. Wash the plate three times with **200 µl** wash buffer.
10. **Vortex** the diluted (1x) SA-PE. Add **50 µl** to each well.
11. Cover and incubate in the dark for **10 min** at RT. Shake at **900–1,100 rpm** for **30 sec** and then turn down to **300–450 rpm** for the specified incubation time.
12. Wash the plate three times with **200 µl** wash buffer.

**13.** Resuspend beads in **125 µl** bead resuspension buffer. Cover and shake at **1,100 rpm** for **30 sec**.

**14.** Read the plate using the appropriate settings as shown below. (**Section 5**)

| Instrument | RP1 (PMT) | DD Gates | Bead Events |
|---|---|---|---|
| Bio-Plex 100, 200* | High | 5,000 (low), 25,000 (high) | 50 |
| Bio-Plex 3D* | Enhanced | Select MagPlex beads | 50 |
| MAGPIX | N/A, use default instrument settings | | |

* Or similar Luminex-based system.





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***Bio-Rad Laboratories, Inc.***

*Life Science Group*

***Web site*** *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800*
***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
***Canada*** *905 364 3435* ***China*** *86 21 6169 8500*
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***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200*
***Sweden*** *08 555 12700* ***Switzerland*** *026 674 55 05*
***Taiwan*** *886 2 2578 7189* ***Thailand*** *800 88 22 88*
***United Kingdom*** *020 8328 2000*