Bio-Plex Pro™ Assays

Quick Guide 2

For Use with	Instruction Manual #
Human, Mouse, and Rat Diabetes Assays	10010747

This guide can be used to prepare and run a full 1 x 96-well assay plate. For more information on a given step, refer to the complete instruction manual. New users can download the manual, which includes detailed instructions and a list of kit components, at **bio-rad.com/bio-plex**.

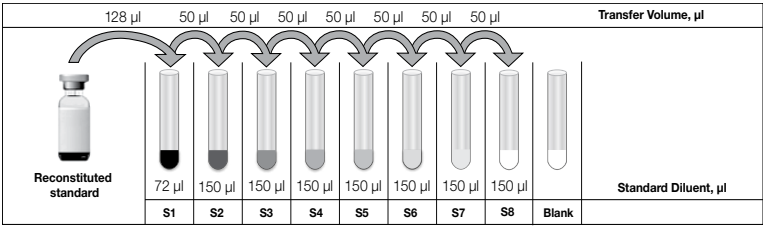
IMPORTANT! Pay close attention to **vortexing**, **shaking**, and **incubation** instructions. Deviation from the protocol may result in low assay signal and assay variability.

Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex® System (**30 min**).
   - Bring assay buffer, wash buffer, and sample diluent to room temperature (RT). Keep other items on ice until needed
   - Begin to thaw frozen samples
3. Prime wash station for flat bottom plate or set vacuum manifold to –1 to –3" Hg for filter plate.
4. Calibrate the Bio-Plex System by following the prompts within Bio-Plex Manager™ Software. This can be done now or during an assay incubation step.
5. Reconstitute a single vial of standards in **500 µl** of a diluent similar to the final sample type or matrix as shown below. **Vortex** for **5 sec** and incubate **on ice** for **30 min**.

Sample Type	Diluent for Standards	Add BSA
Serum and plasma	Standard diluent	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final (w/v)

6. Prepare a fourfold standard dilution series and blank as shown below. **Vortex** for **5 sec** between liquid transfers. If mixing diabetes assays with cytokine assays, refer to the diabetes instruction manual.



7. After thawing samples, prepare as shown below.

Sample Type	Diluent	Add BSA	Sample Dilution
Serum and plasma	Sample diluent	None	Fourfold (1:4)
Culture media, with serum	Culture media	None	Neat to 1:10
Culture media, serum-free	Culture media	To 0.5% final (w/v)	Neat to 1:10
For Adiponectin Assay			
Serum and plasma	Serum-based diluent	None	Human (1:1,600) Mouse (1:1,600) NHP (1:1,600)
For Adipsin Assay			
Serum and plasma	Serum-based diluent	None	Human (1:1,600)

Note: user-defined validation is required for the use of other dilution factors.

8. **Vortex** the 20x coupled beads for 30 sec and dilute to 1x in Bio-Plex Assay Buffer as shown below. Protect from light.

# of Wells	20x Beads, µl	Add BSA	Total Volume, µl
96	288	5,472	5,760

Running the Assay

Note: make sure all assay components are at RT before proceeding.

1. Prewet filter plate with **100 µl** Bio-Plex Assay Buffer (skip for flat bottom).
2. **Vortex** the diluted (1x) beads. Add **50 µl** to each well of the assay plate.
3. Wash two times with **100 µl** Bio-Plex Wash Buffer.
4. **Vortex** samples, standards, blank. Add **50 µl** to each well.
5. Cover plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** for **1 hr** at RT.

Note: 850 rpm provides equivalent performance to recommended shaker settings in previous manuals (**1,100 rpm** for **30 sec**, **300 rpm** for incubation).

6. With 10 min left in the incubation, **vortex** the 20x 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

7. Wash the plate three times with **100 µl** wash buffer.
8. **Vortex** the diluted (1x) detection antibodies. Add **25 µl** to each well.
9. Cover and incubate at **850 ± 50** rpm, as in Step 5, for **30 min** at RT. Meanwhile, prepare Bio-Plex Manager Software protocol; enter standard S1 values provided in the assay kit.
10. With 10 min left in the incubation, **vortex** the 100x 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	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000

11. Wash the plate three times with **100 µl** wash buffer.
12. **Vortex** the diluted (1x) SA-PE. Add **50 µl** to each well.

13. Cover and incubate at **850 ± 50 rpm**, as in Step 5, for **10 min** at RT.

14. Wash the plate three times with **100 µl** wash buffer.

15. Resuspend beads in **125 µl** assay buffer. Cover plate as in Step 5 and shake at **850 ± 50 rpm** for **30 sec**.

16. Remove the sealing tape and **read plate** using the settings below.

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
Bio-Plex® MAGPIX™*	N/A, use default instrument settings	N/A, use default instrument settings	Default

* Or similar Luminex-based system.

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