Bio-Plex Pro Human Diabetes Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Human Diabetes Assays	10000094511

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 go to **bio-rad.com/bio-plex** and download the manual, which includes detailed instructions and a list of kit components.

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 Multiplex Immunoassay System (**30 min**).
   - Bring diluents, including wash buffer, assay buffer, standard diluent HB, detection antibody diluent HB, and sample diluent HB, to room temperature (RT). Keep the other items on ice until needed
     - Mix by inversion to ensure all salts are in solution
     - Prepare 1x wash buffer: dilute **1 part** 10x wash buffer (60 ml) with **9 parts** distilled water (540 ml)
   - Begin to thaw frozen samples
3. After thawing samples, prepare as shown.

Sample Type	Diluent	Add Bovine Serum Albumin (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

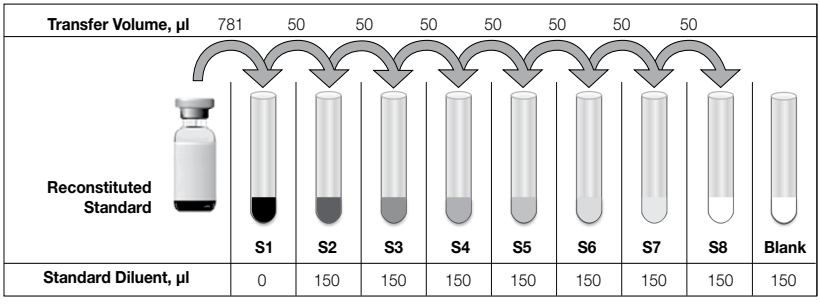
Sample Type	Diluent	Add BSA	Sample Dilution
For Adiponectin Assay			
Serum and plasma	Serum-based diluent	None	Human (1:2,500)
For Adipsin Assay			
Serum and plasma	Serum-based diluent	None	Human (1:2,500)

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

4. Calibrate the Bio-Plex System by following the prompts in Bio-Plex Manager Software. This can be done now or during an assay incubation step.

5. Reconstitute a single vial of standards in **781 µl** of standard diluent HB or a diluent similar to the final sample type or matrix. **Vortex** for **5 sec** and incubate **on ice** for **30 min**.

6. Prepare a fourfold standard dilution series and blank as shown. **Vortex** for **5 sec** between liquid transfers.



7. **Vortex** the coupled beads at medium speed for **30 sec** and **dilute to 1x** in Bio-Plex Assay Buffer as shown. Protect from light.

Premixed Panels

Number of Wells	10x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	570	5,130	5,700

Singleplex Assays

Number of Wells	Singleplex #1 20x Beads, µl	Singleplex #2 20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	285	285	5,130	5,700

Note: 20x singleplex beads allow multiplexing up to 20 analytes.

Running the Assay

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

1. **Vortex** the diluted (1x) beads for a minimum of **10 sec**. Add **50 µl** to each well of the assay plate.
2. **Wash the plate two times** with **100 µl** Bio-Plex Wash Buffer.
3. **Vortex** samples, standards, and blank. Add **50 µl** to each well.
4. Cover the plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** for **1 hr** at RT.
5. With 10 min left in the incubation, **vortex** the detection antibodies for **5 sec** and quick-spin to collect liquid. **Dilute to 1x** as shown.

Premixed Panels

Number of Wells	10x Detection Antibodies, µl	10x Detection Antibody Diluent HB, µl	Total Volume, µl
96	300	2,700	3,000

Singleplex Assays

Number of Wells	Singleplex #1 20x Detection Antibodies, µl	Singleplex #2 20x Detection Antibodies, µl	Detection Antibody Diluent HB, µl	Total Volume, µl
96	150	150	2,700	3,000

Note: 20x singleplex beads allow multiplexing up to 20 analytes.

6. **Wash the plate three times** with **100 µl** wash buffer.
7. **Vortex** the diluted (1x) detection antibodies. Add **25 µl** to each well.
8. Cover and incubate at **850 ± 50 rpm**, as in step 4, for **30 min** at RT. Meanwhile, prepare the Bio-Plex Manager Software protocol; enter standard S1 values provided in the assay kit.
9. With 10 min left in the incubation, **vortex** the 100x streptavidin-phycoerythrin (SA-PE) for **5 sec** and quick-spin to collect liquid. **Dilute to 1x** as shown and protect from light.

Number of Wells	100x SA-PE, µl	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000

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

12. Cover and incubate at **850 ± 50 rpm**, as in step 4, for **10 min** at RT.

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

14. Resuspend the beads in **125 µl** assay buffer. Cover the plate as in step 4 and shake at **850 ± 50 rpm** for **30 sec**.

15. Remove the sealing tape and **read plate** using the following settings:

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 200*	Low	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 System.

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