Bio-Plex Pro Rat Diabetes Assays

Quick Guide

For Use with	Instruction Manual #
Rat Diabetes Assays	10000094509

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.

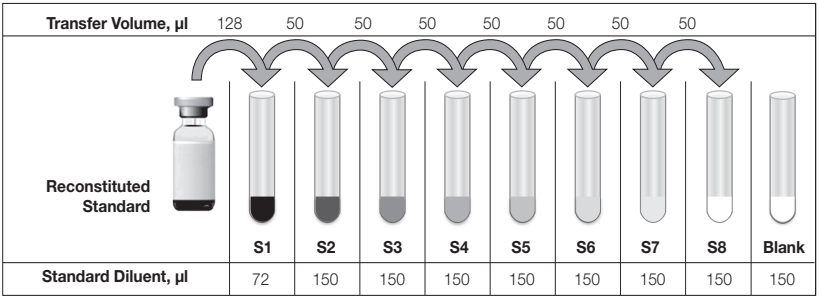
IMPORTANT! Pay close attention to **vortexing**, **shaking**, and **incubation** instructions. Deviation from the protocol may result in low assay signal and assay variability.

Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex 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
   - Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Then dilute 1 part 10x wash buffer (60 ml) with 9 parts distilled water (540 ml)
3. Prime the wash station for flat bottom plate.
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 **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 Bovine Serum Albumin (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.



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

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.

Number of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5,472	5,760

Running the Assay

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

1. **Vortex** the diluted (1x) beads. 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 20x detection antibodies for **5 sec** and quick-spin to collect liquid. Dilute to 1x in detection antibody diluent as shown below.

Number of Wells	20x Detection Ab, µl	Detection Ab Diluent HP, µl	Total Volume, µl
96	150	2,850	3,000

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 the plate and incubate at **850 ± 50 rpm**, as in step 4, for **30 min** at RT. Meanwhile, prepare 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 below 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 the plate 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 beads in **125 µl** assay buffer. Cover plate as in step 4 and shake at **850 ± 50 rpm** for **30 sec**.

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

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

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