Bio-Plex Pro™ RBM IGF and IGFBP Assays

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro™ RBM IGF and IGFBP Assays	10042015

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 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.

A. Reagent Preparation

1. Reconstitute the following lyophilized reagents in dH_20 before use, according to the table below.

Reagent	Volume, µl
Standards Mix	150
Control 1	100
Control 2	100

Reagent	Volume, ml
Blocking Buffer	1.5
Standard Diluent	1.0
Detection antibodies	4.8

a. Allow vial to sit at room temperature for a minimum of **5 min**, not to exceed **30 min**.

b. Mix by **vortexing** at a medium setting.

2. Bring the 10x assay buffer to ambient/room temperature (RT).

a. Mix by inversion to ensure all salts are in solution.

b. Prepare 1x assay buffer — dilute **1 part** 10x assay buffer (60 ml) with **9 parts** of dH_20 (540 ml).

B. Dilution of Standard (1:3 Serial Dilution)

1. Label 9 polypropylene tubes **S1** through **S8** and **Blank**.

2. Transfer the reconstituted standard into the tube labeled **S1**.

3. Add the appropriate amount of the standard diluent into the labeled tubes according to the table below (this will be sufficient for duplicate standard curves and blanks).

Standard	Volume of Standard Diluent, µl	Volume of Standard, µl
S1	—	150 from reconstituted vial
S2	100	50 of S1
S3	100	50 of S2
S4	100	50 of S3
S5	100	50 of S4
S6	100	50 of S5
S7	100	50 of S6
S8	100	50 of S7
Blank	100	—

4. Prepare working standards (**S2–S8**) by serial dilution. Transfer the appropriate volume of standard into each of the labeled tubes with standard diluent, as outlined above.

5. **Vortex** each standard at a medium setting before proceeding with the next serial dilution. Change pipet tip at each dilution step.

6. The **Blank** tube consists of standard diluent alone.

C. Sample Preparation

Note: Most of the circulating IGF-1 and IGF-2 are in complex with IGFBPs. A sample extraction step is required prior to measuring IGFs in serum and plasma samples. Refer to **Section 5** of the instruction manual for guidance on extraction protocol. The IGF-1 and IGF-2 assays have been optimized to measure total IGF. For users measuring free IGF, please skip the sample extraction step and proceed to dilute the sample 1:5 with the provided sample diluent. The IGFBP assays do not require sample extraction.

1. Centrifuge serum or plasma samples at **1,000 x g** for **15 min** at 4°C to remove particulates from all samples prior to use. Refer to instruction manual #10042015 for a detailed sample extraction protocol.

2. Prepare sample dilutions in **0.5 ml** or **1.0 ml** polypropylene tubes, as required for the assay.

3. Dilution scenarios provided below are sufficient to run each sample in duplicate.

Panel	Sample Dilution	Volume of Sample	Volume of Extraction Buffer	Volume of Neutralization Buffer	Volume of Sample Dilution Buffer 2
IGF	1:30	20 µl	180 µl	Mix 50 µl of extracted sample with 50 µl of neutralization buffer	Mix the neutralized sample with 50 µl of sample dilution buffer 2
IGFBP	1:20	10 µl	N/A	N/A	190 µl

Note: Controls are ready to use after reconstitution. No further dilution is needed.

D. Dispensing of Reagents

Note: This protocol is optimized with a single wash step after the SA-PE incubation. Do not wash the plate after the sample incubation and the detection antibody incubation steps.

1. Add **10 µl** of blocker to all wells of the plate.
2. Add **30 µl** of the standard, control, sample, or blank to the appropriate well of the plate.
3. **Vortex** the capture beads at medium speed for **10–20 sec**. Add **10 µl** of the beads to all wells of the plate.
4. Cover plate with plate seal and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** for **1 hr** at RT. **Do not aspirate after incubation.**
5. **Vortex** the reconstituted detection antibodies at medium speed for **10–20 sec**. Add **40 µl** to each well.
6. Cover and incubate at **850 ± 50 rpm**, as in step 4, for **1 hr** at RT. **Do not aspirate after incubation.**
7. Prepare the required dilution of streptavidin-PE (SA-PE), as outlined in the following table.

   Note: Volumes in the table are for an entire 96-well plate. Smaller volumes can be prepared, provided that the dilution ratios are maintained.

8. Add **20 µl** of diluted SA-PE to the required plate wells.

SA-PE Dilution	Volume of SA-PE, µl	Volume of 1x Assay Buffer, µl	Total Volume, µl
1:10	225	2,025	2,250

9. Cover and incubate at **850 ± 50 rpm**, as in step 4, for **30 min** at RT.

10. Wash the plate three times with **100 µl** 1x assay buffer.

11. After the final wash, resuspend the beads in **100 µl** 1x assay buffer. Cover plate, as in step 4, and shake the plate at **850 ± 50 rpm** for **30 sec**.

12. Remove the plate seal and **read plate** at low PMT (Bio-Plex® 200), standard PMT (Bio-Plex 3D), or default settings (Bio-Plex® MAGPIX™).

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