Bio-Plex Pro™ RBM Apoptosis Assays

Quick Guide

For Use With	Instruction Manual #
Bio-Plex Pro™ RBM Apoptosis Assays	10033631

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_2O 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 temperature (RT).

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

b. Prepare 1x assay buffer — dilute **1 part** 10x assay buffer (60 ml) with **9 parts** of dH_2O (540 ml).

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

1. Label 8 polypropylene tubes **S1** through **S8**.

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
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.

C. Sample Preparation

1. Refer to instruction manual #10033631 for detailed sample preparation.

Note: Pay close attention to cell lysis, homogenization, and fractionation protocols in the manual. Analytes of interest may be localized to the cytosolic fraction or to the nuclear and mitochondrial fraction.

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

3. For sample analysis, bring samples to a final protein concentration of **500 µg/ml** by diluting with lysis dilution buffer (LDB). Further dilution may be necessary for targets with high expression levels.

4. Centrifuge samples at **500 x g** for **5 min** to remove particulates prior to use.

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

D. Dispensing of Reagents

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.

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

6. **Vortex** the reconstituted detection antibodies at medium speed for **10–20 sec**. Add **40 µl** to each well.

7. Cover and incubate at **850 ± 50 rpm**, as in Step 4, for **1 hr** at RT. **Do not aspirate after incubation.**

8. 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.

9. 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

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

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

12. 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**.

13. 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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Bio-Plex Pro RBM kits are manufactured by Myriad RBM.

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