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** 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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Life Science Group

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Bio-Plex Pro™ RBM Kidney Toxicity Assays

Quick Guide

For Use With	Instruction Manual #
Bio-Plex Pro™ RBM Human, Rat, and Canine Kidney Toxicity Assays	10028258

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 room temperature (RT).

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

b. Prepare 1x assay buffer — dilute **1 part** 10x assay buffer with **9 parts** of dH_20.

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

Bio-Plex Pro Assay Quick Guide

Panel	Sample Dilution	Volume of Urine Sample, µl	Volume of Sample Buffer, µl
Human Tox 1	1:4	20	60
Human Tox 2	1:50	10	490
Rat Tox 1	1:2	40	40
Rat Tox 2	1:50	10	490
Rat Albumin	1:10,000	5 (A. Prepare 1:100) 5 (B. Prepare 1:100)	495 495
Canine Tox 1	1:15	10	140
Canine Albumin	1:10,000	5 (C. Prepare 1:100) 5 (D. Prepare 1:100)	495 495

Note: Controls are ready to use after reconstitution. No 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 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 dilution ratios are maintained.

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