Bio-Plex Pro™ Human Apolipoprotein 10-Plex Assay

Quick Guide

For Use with	Instruction Manual #
Bio-Plex Pro Human Apolipoprotein 10-Plex Assay	10000071714

This quick 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 Bio-Plex Pro Human Apolipoprotein 10-Plex Assay Instruction Manual (#10000071714). Go to bio-rad.com/web/bio-plex to download the instruction 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.

Sample Preparation

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.
2. Prepare sample dilutions in **0.5 ml** or **1.0 ml** polypropylene tubes as required for the assay.
3. Prepare the sample dilution as outlined in Table 1. The dilution example in Table 1 provides a sufficient amount to run each sample in duplicate.

Table 1. Preparation of the sample dilutions.

Sample Dilution	Volume of Sample, µl		Volume of Sample Diluent, µl
1:50,000	(a) Prepare 1:10	10	90
	(b) Prepare 1:50	10 (a)	490
	Prepare 1:100	5 (b)	495

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

Reagent Preparation

1. Reconstitute the following lyophilized reagents in distilled water (dH_2O) before use as outlined in Table 2.

Table 2. Reconstitution of the lyophilized reagents.

Reagent	Volume dH_2O
Standards mix	150 µl
Control 1	100 µl
Control 2	100 µl
Blocking buffer	1.5 ml
Standard diluent	1.0 ml
Detection antibodies	4.8 ml

   a. Allow vial to sit at room temperature for **5 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 in solution.

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

Dilution of Standard (1:3 serial dilution)

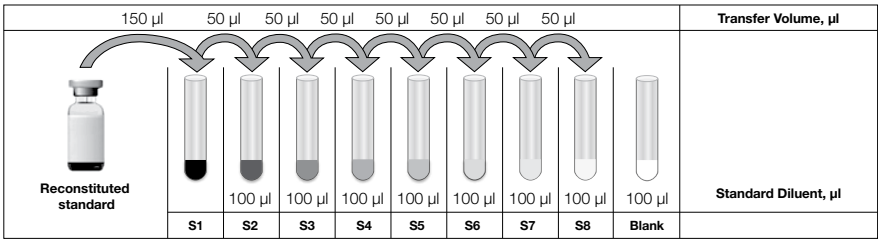
1. Label 9 polypropylene tubes S1 through S8 and Blank.

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

3. Add the appropriate amount of standard diluent into the labeled tubes as outlined in Table 3 (this will be sufficient for duplicate standard curves and blanks).

Table 3. Dilution of the standards.

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 the pipet tip at each dilution step.
6. The **Blank** tube consists of standard diluent alone.

Dispensing 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.

   Note: A multichannel pipet is recommended. If using a single channel pipet, vortex the beads frequently to keep them in solution.
4. Cover the plate with a plate seal and protect it from light with aluminum foil. Incubate on a shaker at **850 ± 50 rpm** for **1 hr** at RT.
5. Wash the plate three times with **100 µl** per well of 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 the plate 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-phycoerythrin (SA-PE) as outlined in Table 4.

   Note: Volumes in Table 4 are for an entire 96-well plate. Different volumes can be prepared, provided that the dilution ratios are maintained.

Table 4. Dilution of SA-PE.

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. Add **20 µl** of diluted SA-PE to each plate well.
10. Cover and incubate the plate at **850 ± 50 rpm**, as in Step 4, for **30 min** at RT.
11. Wash the plate three times with **100 µl** per well of 1x assay buffer.
12. After the final wash, resuspend the beads in each assay well with **100 µl** 1x assay buffer. Cover the plate as in Step 4 and shake the plate at **850 ± 50 rpm** for **30 sec**.
13. Remove the plate seal and read the plate at low photomultiplier tube (PMT) (Bio-Plex® 200 System), standard PMT (Bio-Plex 3D Suspension Array System), or default setting (Bio-Plex® MAGPIX™ Multiplex Reader).

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