Bio-Plex Pro™ Human TIMP Assays

Quick Guide

For use with	Instruction Manual #
Bio-Plex Pro Human TIMP* Assays	10041640

* TIMP, tissue inhibitor of metalloproteinase

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 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.

Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex® system (**30 min**).
   - Bring diluents including wash buffer, assay buffer, diluent HD, and detection antibody diluent HB to room temperature (RT). Keep other items on ice until needed
   - Begin to thaw frozen samples
3. Prime wash station for flat bottom plate or set vacuum manifold to –1 to –3" Hg for filter plate.
4. Calibrate the Bio-Plex system by following the prompts within the Bio-Plex Manager™ software. This can be done now or during an assay incubation step.
5. Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Then dilute 1 part 10x wash buffer with 9 parts dH_2O.

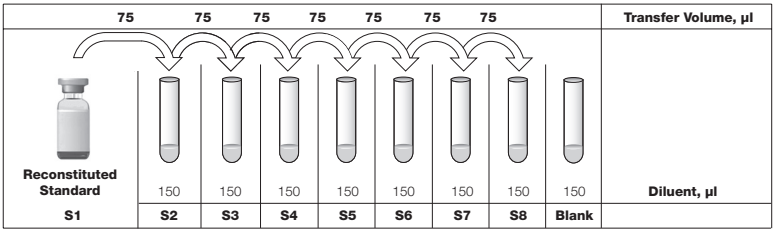
6. Reconstitute the vial of standards in **781 µl** of diluent HD (or a diluent similar to your sample matrix). Reconstitute the vial of control in **250 µl** of the same diluent, as shown below. **Vortex** at medium speed for **5 sec** and incubate all vials **on ice** for precisely **30 min**.

Sample Type	Diluent for Standards and Controls*	Add BSA
Serum and plasma	Diluent HD	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final

* If using diluents other than the diluent HD provided, then users must establish their own control ranges.

7. Prepare a threefold standard dilution series and blank as shown below. **Vortex** at medium speed for **5 sec** between liquid transfers.

Note: The controls are ready to use. No dilution is needed.



8. After thawing samples, prepare according to the guidelines shown below.

Sample Type	Dilution Factor*	Recommended Sample Dilution
Serum and plasma	TIMP: 1:50 dilution	Diluent HD
Fluids	TIMP: 1:4 and 1:40	Diluent + 0.5% BSA w/v

* TIMP-3 is for tissue culture samples only.

9. Vortex coupled beads for **30 sec** and dilute to 1x in Bio-Plex assay buffer as shown below. Protect from light.

# 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 pipetting. **Vortex** at medium speed.

1. Prewet filter plate with **100 µl** Bio-Plex assay buffer (skip for flat bottom).
2. **Vortex** the diluted (1x) beads. **Add 50 µl** to each well of the assay plate.
3. **Wash the plate two times** with **100 µl** Bio-Plex wash buffer.
4. **Vortex** samples, standards, blank, and control. **Add 50 µl** to each well.
5. Cover plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** at RT for **1 hr**.
6. With 10 min left in the incubation, **vortex** detection antibodies for **15 sec** and quick-spin to collect liquid. Dilute to 1x as shown below.

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

7. **Wash the plate three times** with **100 µl** wash buffer.
8. **Vortex** the diluted (1x) detection antibodies. **Add 25 µl** to each well.
9. **Cover and incubate** at **850 ± 50 rpm**, as described above, in the dark for **30 min** at RT. Meanwhile, prepare Bio-Plex Manager software protocol; enter standard S1 values and units provided in the assay kit.
10. With 10 min left in the incubation, **vortex** 100x streptavidin-PE (SA-PE) for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown below and protect from light.

# of Wells	100x SA-PE, µl	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000

11. **Wash the plate three times** with **100 µl** wash buffer.
12. **Vortex** the diluted (1x) SA-PE. **Add 50 µl** to each well.
13. **Cover and incubate** at **850 ± 50 rpm**, as described above, in the dark for **10 min** at RT.

14. **Wash the plate three times** with **100 µl** wash buffer.

15. Resuspend beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec**.

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

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 100, 200*	Low	5,000 (low); 25,000 (high)	50
Bio-Plex 3D*	Standard	Select MagPlex beads	50
Bio-Plex® MAGPIX™	N/A, use default instrument settings		

* A similar Luminex-based system may be used.

17. Compare the observed concentration against the ranges provided in the assay kit. Ranges apply only when standards and controls are prepared in Bio-Plex diluent HD.

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