BIO-RAD

Bio-Plex Pro™ Human Th17 Cytokine Assays

Quick Guide

For Use with*	Instruction Manual #
Bio-Plex Pro Human Th17 Cytokine Panel	10023381

* Includes premixed multiplex panels, singleplex sets, custom x-Plex™ Assays, and Express Assays.

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 **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

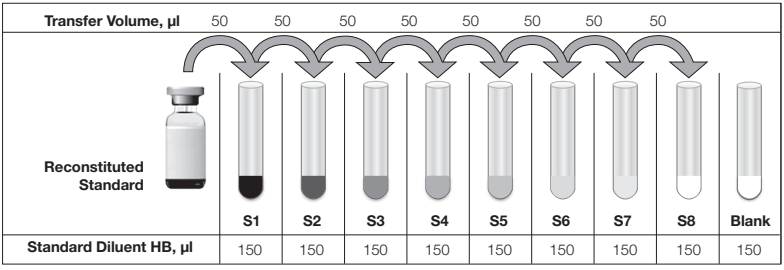
1. Plan the plate layout (**Section 1** of the complete instruction manual).
2. Start up/warm up the Bio-Plex® System (**30 min**) (**Section 2**).
   - Meanwhile, bring buffers and diluents to room temperature (RT)
   - Begin to thaw frozen samples
   - Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Then dilute 1 part 10x wash buffer (60 ml) in 9 parts dH_20 (540 ml)
3. Prime wash station (**Section 3**).
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 (**Section 2**).
5. Reconstitute a single vial of standard in **781 µl** of a diluent similar to the final sample type or matrix. Reconstitute the quality controls in **250 µl** of the same diluent, as shown below. **Vortex** for **5 sec** and incubate all vials at once **on ice** for **30 min** (**Section 4**).

   Note: The quality controls are ready to use after reconstitution; no dilution is needed. Only premixed panels and x-Plex™ Kits include controls.

Sample Type	Diluent for Standard and Controls*	Add BSA
Serum and plasma	Standard diluent HB	None
Culture media, with serum	Culture media	None
Culture media, serum-free	Culture media	To 0.5% final
Lavage, lysate, other fluids	Sample diluent HB	To 0.5% final

* If using diluents other than standard diluent HB, users must establish their own control ranges.

6. Prepare a fourfold standard dilution series and blank as shown below. **Vortex** for **5 sec** between liquid transfers (**Section 4**).



* Use undiluted reconstituted standard as S1.

7. After thawing samples, prepare as shown below (**Section 5**).

Sample Type	Diluent	Add BSA	Sample Dilution
Serum and plasma	Sample diluent HB	None	Fourfold (1:4)
Culture media, with serum	Culture media	None	Neat to 1:10
Culture media, serum-free	Culture media	To 0.5% final	Neat to 1:10
Lavage, lysate, other fluids	Sample diluent HB	To 0.5% final	User optimized

8. Vortex coupled beads for **30 sec** and dilute to 1x in Bio-Plex Assay Buffer as shown below. Protect from light (**Section 6**).

# of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5,472	5,760

Running the Assay (all **Section 7** in the manual unless otherwise noted)

Note: make sure all assay components are at RT before proceeding.

1. **Vortex** the diluted (1x) beads. **Add 50 µl** to each well of the assay plate.
2. **Wash the plate two times** with **100 µl** Bio-Plex Wash Buffer.
3. **Vortex** samples, standards, blank. **Add 50 µl** to each well.
4. **Cover and incubate** in the dark for **1 hr** at RT with vigorous shaking.

   Note: Shake at **850 ± 50 rpm**. Ramp up to speed slowly to avoid splashing.
5. With 10 min left in the incubation, **vortex** detection antibodies for **5 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	145	2,755	2,900

* Th17 assay uses detection Ab diluent HB.

6. **Wash the plate three times** with **100 µl** wash buffer.
7. **Vortex** the diluted (1x) detection antibodies. **Add 25 µl** to each well.
8. **Cover and incubate** in the dark for **30 min** at RT. Shake at **850 ± 50** rpm. Meanwhile, prepare Bio-Plex Manager Software protocol; enter standard S1 values provided in the assay kit (**Section 8**).
9. With 10 min left in the incubation, **vortex** 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

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

14. Resuspend beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec** (**Section 8**).

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

* Or similar Luminex based system.

15. If quality controls were run, compare the observed concentrations against the ranges provided in the assay kit. Ranges apply only when standard and controls are prepared in Bio-Plex Standard Diluent HB.

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10000080121 Ver B (10022581) US/EG *18-0308 0418 Sig 0118*