

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
 - 40ml 5x Assay wash buffer
 - 160ml ddH₂O
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
(AVOID FREEZE/THAW OF ANTIBODY MIX)
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100ul directly to the well or dilute 2-fold with 1X Diluent buffer.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual on our website.
- For **serum or plasma samples**, we recommend a 1:10 to 1:20 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare 3.5ml sample in 1X Diluent buffer and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
Optional: If you want to have a blank reading, you can design one well as a blank well by adding diluent buffer instead of your sample.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
9. Add 100µl substrate to each well and incubate for 30-40 minutes at least.

Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes. Always stop the reaction of samples from the same row at the same time.

10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Diagram of Mouse Cytokine ELISA Plate Array III

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNF α	IL-1 α	PDGF-BB	CCL11	TNF α	IL-1 α	PDGF-BB	CCL11	TNF α	IL-1 α	PDGF-BB	CCL11
B	IGF-1	IL-1 β	β -NGF	CCL21	IGF-1	IL-1 β	β -NGF	CCL21	IGF-1	IL-1 β	β -NGF	CCL21
C	VEGF	G-CSF	IL-17a	IL-3	VEGF	G-CSF	IL-17a	IL-3	VEGF	G-CSF	IL-17a	IL-3
D	IL-6	GM-CSF	IL-2	IL-13	IL-6	GM-CSF	IL-2	IL-13	IL-6	GM-CSF	IL-2	IL-13
E	FGFb	MCP-1	IL-4	IL-21	FGFb	MCP-1	IL-4	IL-21	FGFb	MCP-1	IL-4	IL-21
F	IFN γ	M1P-1 α	IL-10	IL-22	IFN γ	M1P-1 α	IL-10	IL-22	IFN γ	M1P-1 α	IL-10	IL-22
G	EGF	SCF	Resistin	CXCL10	EGF	SCF	Resistin	CXCL10	EGF	SCF	Resistin	CXCL10
H	Leptin	Rantes	IL-12	CXCL1	Leptin	Rantes	IL-12	CXCL1	Leptin	Rantes	IL-12	CXCL1