Luminex® Extracellular Assay Protocol

Before getting started visit www.invitrogen.com/luminex to complete Calculation Worksheet and Plate Planner.

Wash Solution Preparation

Prepare 1x Working Wash Solution by diluting the entire contents of the 20x Wash Solution bottle with 285 ml ddH_20.

Assay Standard Preparation

Reconstitute the lyophilized standard in 100% Assay Diluent (serum and plasma samples) or 50% Assay Diluent/50% tissue culture media (tissue culture supernatants):

Reconstitution Volumes:

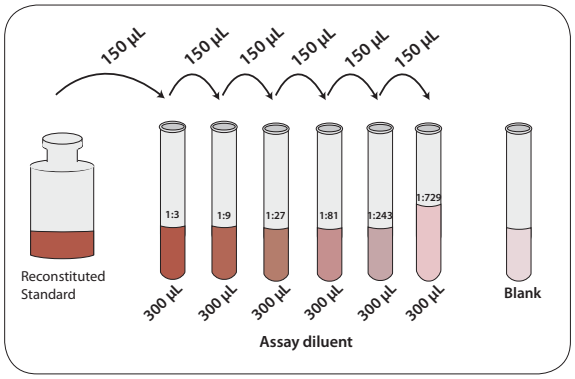
(confirm reconstitution volumes on technical data sheet included with the kit)

- 1 vial: 1 ml
- 2 vials: 0.5 ml per vial
- 3 vials: 0.333 ml per vial
- 4 vials: 0.25 ml per vial

Rehydrate at room temperature for 8-10 minutes. Gently invert the vial(s) several times and let sit an additional 3-5 minutes to ensure complete hydration. If more than 1 standard is used, combine equal volumes of each standard and gently mix.

Perform 3-fold serial dilutions of the reconstituted standard to prepare a seven point standard curve.

Figure 1—Serial dilution.



Analyte Capture

1. Vortex (30 sec) and sonicate (30 sec) the 10x Capture Bead stock. In a foil wrapped tube, dilute the 10x Capture Bead stock (2.5 µl per well) in Working Wash Solution (25 µl per well). For higher multiplexing adjust the volume of Working Wash Solution to account for the extra volumes of 10x Capture Bead stocks retained. (Refer to Calculation Worksheet).

   NOTE: The human 30-plex, 25-plex and mouse 20-plex capture beads are supplied at 1x and are ready to use (25 µl per well).

2. Pre-wet the standard and sample wells with 200 µl Working Wash Solution.

   NOTE: All assay steps are performed in a 96-well filter plate. Remove liquid from the plate with a vacuum manifold (Do not exceed 10 In. of Hg). Never turn the plate over. If clogging should occur, use the pointed end of a 15 ml conical tube to gently press the area under the clogged well and then use a 1 ml Pasteur pipette rubber bulb or place thumb over clogged well to dislodge clog by generating pressure. Following final aspiration step, lightly tap bottom of plate on a stack of paper towels and then dab the bottom of the filter plate with a Kimwipe to remove residual liquid/droplets.

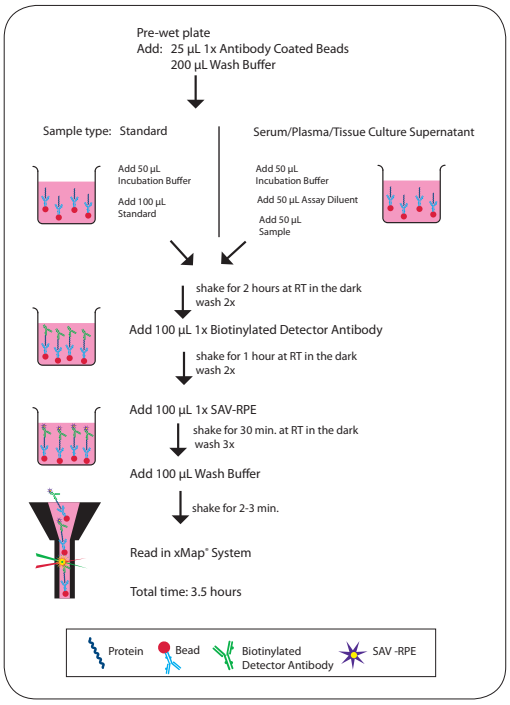
3. Vortex (30 sec) and sonicate (30 sec) the diluted Capture Bead solution. Immediately add 25 µl to each assay well followed by 200 µL of 1x Wash Solution. Aspirate and repeat the wash with 200 µL of Working Wash Solution. Tap and dab the bottom of the filter plate as needed.

4. Add 50 µl Incubation Buffer to all assay wells.

5. Add 100 µl standard into designated wells. For wells designated for samples, add 50 µl Assay Diluent followed by 50 µl sample. Cover and incubate the plate for 2 hours at room temperature on an orbital plate shaker (500-600 rpm).

   NOTE: Cover the assay plate with an opaque lid during all incubations to protect from light. The speed may need to be adjusted depending upon the radius of the orbital shaker.

Figure 3—General assay protocol summary.



For a complete list of multiplex products, visit www.invitrogen.com/luminex.

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Analyte Detection

6. Prepare 1x Biotinylated Detector Antibody: Dilute the 10x Biotinylated Detector Antibody (10 µl per well) in Biotin Diluent (100 µl per well). For higher multiplexing, adjust the volume of Biotin Diluent to account for the extra volumes of 10x Biotinylated Antibody stocks required. Aspirate and wash the assay wells twice with 200 µl Working Wash Solution. Add 100 µl diluted Biotinylated Detector Antibody to each assay well. Cover and incubate the plate for 1 hour on a plate shaker (500-600 rpm).

7. Prepare 1x Streptavidin-RPE solution: Dilute the 10x Streptavidin-RPE (10 µl per well) in RPE-Diluent (100 µl per well) in a foil wrapped tube. Aspirate and wash the assay wells twice with 200 µl Working Wash Solution. Add 100 µl diluted Streptavidin-RPE to each assay well. Cover and incubate the plate for 30 minutes on a plate shaker (500- 600 rpm).

Assay Reading

8. Aspirate and wash the assay wells 3 times with 200 µl Working Wash Solution. Dry the bottom of the filter plate with clean paper towels to completely remove all residual droplets. Add 100 µl Working Wash Solution to each assay well and place the plate on the plate shaker (500-600 rpm) for 2-3 minutes.

9. Read the plate on any Luminex® 100™ or 200™ instrument. Please refer to the complete protocol booklet (Appendix II) for the appropriate instrument settings for BioSource™ Antibody Bead Assays.

Table 1—Extracellular bead region reference chart.

Marker	Bead Region	Marker	Bead Region	Marker	Bead Region
Adiponectin	14	IL-2	54	Leptin	32
Aggregated Aβ	60	IL-2R	61	MCP-1/CCL2	29
Aggregated α-Synuclein	76	IL-3	53	MCP-2/CCL8	52
Aβ40	60	IL-4	77	MCP-3/CCL7	48
Aβ42	60	IL-5	34	MIG/CXCL9	63
BDNF	57	IL-6	19	MIP-1α/CCL3	26
C-Reactive Protein	39	IL-6 US	19	MIP-1β/CCL4	28
DR5	37	IL-6R	36	MIP-3β	16
EGF	08	IL-7	55	PDGF-BB	40
Eotaxin/CCL11	22	IL-8	81	RANTES/CCL5	21
FGF basic	12	IL-9	44	Resistin	35
G-CSF	07	IL-10	hu 9/ rt15	Serum Amyloid A (SAA)	33
GDNF	58	IL-12 (p40/p70)	20	Tau (total)	69
GM-CSF	27	IL-12 (p70)	hu 42/ ms 20	Tau [pT181]	69
GRO-α	45	IL-13	23	Tau [pS199]	69
HGF	10	IL-15	24	TGF-β1 (activated/treated)	41
IFN-α	17	IL-16	43	TNF-RI	13
IFN-γ	38	IL-17	25	TNF-RII	11
IL-1α	04	Insulin	31	TNF-α	50
IL-1β	06	IP-10	56	VEGF	05
IL-1RA	51	KC	41		