User Guide

MILLIPLEX® PLEXpedition Magnetic Bead Screening Panel

96-Well Plate Assay

HPLX1-115SP, HPLX1-115SP-PX, HPLX1-115SP-PX80

Introduction

The MILLIPLEX® PLEXpedition Screening Panel is a configurable multiplex immunoassay kit developed to simultaneously screen up to 115 targets including cytokines, chemokines, growth factors, matrix metalloproteinases (MMPs), and biomarkers of bone health, metabolism, and cardiovascular disease. Two Luminex® instruments, the xMAP® INTELLIFLEX and the FLEXMAP 3D®, can analyze all 115 bead regions with comparable sensitivity and sample correlation while the MAGPIX® and Luminex® 200™ instruments can detect specific subsets of 50 and 80 analytes, respectively. As a discovery biomarker tool this panel is provided in a matrix-free format to enable analyte detection in multiple sample types. After screening with PLEXpedition to identify markers of interest, customers can transition to our Qualified MILLIPLEX® panels, each of which has an optimized serum matrix and lot-to-lot verification for reproducible quantification of protein concentration.

The MILLIPLEX® portfolio offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. Coupled with the Luminex® xMAP® platform in a magnetic bead format, with each assay you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

With MILLIPLEX® Qualified and Screening Assays you can select a biomarker solution fit for your purpose from the most versatile system available for research across multiple therapeutic areas.

MILLIPLEX® Screening Assays

- Contain all reagents necessary to conveniently run the assay including premixed beads and premixed detection antibodies
- Verify analyte specificity across the entire panel
- Are sample agnostic for greater flexibility
- Enable easy transition to Qualified Assays by utilizing same critical raw materials

The MILLIPLEX® PLEXpedition Magnetic Bead Screening Panel is a 115-plex kit to be used for the simultaneous quantification of any or all of the following analytes in your selected sample type: ACTH, Active Ghrelin, Amylin Active, BCA-1, BDNF, BNP, CK-MB, C-Peptide, CXCL9/MIG, DKK1, EGF, ENA-78, Eotaxin/CCL11, Eotaxin-2, Eotaxin-3, Erythropoeitin (EPO), FABP3, FABP4, FGF-2, FGF-21, FGF-23, FLT3 Ligand, Fractalkine/CX3CL1, G-CSF, GIP, GLP-1 Total, Glucagon, GM-CSF, GCP-2/CXCL6, Granzyme A, Granzyme B, GROα, HB-EGF, HGF, I-309, IFNα2, IFNγ, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A/CTLA8, IL-17F, IL-18, IL-1RA, IL-1α, IL-1β, IL-2, IL-21, IL-22, IL-23, IL-3, IL-33, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, Insulin, IP-10/CXCL10, I-TAC, Leptin, LIF, MCP-1/CCL2, MCP-2, MCP-3/CCL7, MCP-4, M-CSF, MDC/CCL22, MIP-1α/CCL3, MIP-1β/CCL4, MIP-1δ, MIP-3α, MIP-3β, MMP-1, MMP-10, MMP-12, MMP-2, MMP-3, MMP-7, MMP-9, Myostatin (MSTN)/GDF8, NGF, NTproBNP, Osteocalcin (OC), OPG, OPN, Osteonectin, PDGF-AA, PDGF-AB/BB, Perforin, PLGF, PP, PYY, RANTES/CCL5, sCD40L, SCF, SDF-1, sFas, sFasL, SOST, TARC, TGFα, TNFα, TNFβ/LTA, TPO, TRAIL, Troponin I, TSLP, VEGF-A, VEGF-C, VEGF-D.

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Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

Principle

MILLIPLEX® kits are based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex® microspheres.

- Luminex® uses proprietary techniques to internally color-code microspheres with three fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500 5.6 µm polystyrene microspheres or 6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The following Luminex® instruments can be used to acquire and analyze data using two detection methods:
- The Luminex® analyzers Luminex® 200™, FLEXMAP 3D®, and xMAP® INTELLIFLEX are flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics, and high-speed digital signal processors.
- The Luminex® analyzer, MAGPIX®, is a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified, and the result of its bioassay is quantified based on fluorescent reporter signals. We combine the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of MILLIPLEX® Belysa®, integrating data acquisition and analysis seamlessly with all Luminex® instruments.
- xMAP® INTELLIFLEX runs on INTELLIFLEX software for instrument control, run setup and generating high quality data with flexible output options. Data can be exported in xPONENT® style CSV files for compatibility with many existing analytical applications, or in the new, customizable INTELLIFLEX file format. The INTELLIFLEX file format is intended for flexibility and simplicity, allowing the user to freely select which data points to include and to reduce the time to analysis.
- The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

Storage Conditions Upon Receipt

- Recommended storage for kit components is 2-8 °C.
- For long-term storage, freeze reconstituted standards at ≤ -20 °C. Avoid multiple (> 2) freeze/thaw cycles.
- DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.

Reagents Supplied

MILLIPLEX® PLEXpedition Standard Cocktails (10X)

Reagents	Volume	Quantity	Catalogue No.
PLEXpedition Standard 1 (10X)	Lyophilized	1 vial	HPLX1-9010-01
PLEXpedition Standard 2 (10X)	Lyophilized	1 vial	HPLX1-9010-02
PLEXpedition Standard 3 (10X)	Lyophilized	1 vial	HPLX1-9010-03
PLEXpedition Standard 4 (10X)	Lyophilized	1 vial	HPLX1-9010-04
PLEXpedition Standard 5 (10X)	Lyophilized	1 vial	HPLX1-9010-05
PLEXpedition Standard 6 (10X)	Lyophilized	1 vial	HPLX1-9010-06
PLEXpedition Standard 7 (10X)	Lyophilized	1 vial	HPLX1-9010-07

Included MILLIPLEX® PLEXpedition Standard Cocktails (10X) are dependent on customizable selection of analytes within the panel.

MILLIPLEX® PLEXpedition Screening Panel Detection Antibodies

Reagents	Volume	Quantity	Catalogue No.
PLEXpedition Detection Antibody	3 mL	1 bottle	HPLX1-2010
PLEXpedition Custom Premixed Detection	3 mL	1 bottle	-

Included MILLIPLEX® PLEXpedition Screening Panel Detection antibodies are dependent on customizable selection of analytes within the panel.

Reagents Included Independent of Analyte Selection

Reagents	Volume	Quantity	Catalogue No.
Set of one 96-Well Plate with 2 sealers	-	1 plate 2 sealers	-
Assay Buffer	30 mL	1 bottle	L-AB
10X Wash Buffer	60 mL	1 bottle	L-WB
Streptavidin-Phycoerythrin	3 mL	1 bottle	HPLX-SAPE

MILLIPLEX® PLEXpedition Screening Panel Antibody-Immobilized Premixed Magnetic Beads

Reagents	Volume	Quantity	Catalogue No.
PLEXpedition 115-plex Bead Mix (1X)	3 mL	1 bottle	HPLX1BM115-MG
PLEXpedition 80-plex Bead Mix (1X)	3 mL	1 bottle	HPLX1BM80-MG
Custom Premixed Beads	3 mL	1 bottle	--

Included MILLIPLEX® PLEXpedition Screening Panel Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see below).

		Available Panel		Instrument Compatibility		
Analyte	Luminex® Bead Region	Fixed 80plex	Fixed or configurable 115plex	MAGPIX®	Luminex® 200™	FLEXMAP 3D® or xMAP® INTELLIFLEX
Amylin (Active)	1		✓			✓
BCA-1	2		✓			✓
BNP	3		✓			✓
CK-MB	4		✓			✓
GCP-2	5		✓			✓
DKK1	6		✓			✓
ACTH	7	✓	✓		✓	✓
BDNF	8	✓	✓		✓	✓
C-Peptide	9	✓	✓		✓	✓
ENA-78	10		✓			✓
Eotaxin-2	11		✓			✓
EGF	12	✓	✓	✓	✓	✓

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Analyte	Luminex® Bead Region	Available Panel		Instrument Compatibility		
		Fixed 80plex	Fixed or configurable 115plex	MAGPIX®	Luminex® 200™	FLEXMAP 3D® or xMAP® INTELLIFLEX
FGF-2	13	✓	✓	✓	✓	✓
Fractalkine	14	✓	✓	✓	✓	✓
G-CSF	15	✓	✓	✓	✓	✓
Eotaxin-3	16		✓			✓
Erythropoeitin (EPO)	17		✓			✓
Ghrelin (Active)	18	✓	✓	✓	✓	✓
GIP	19	✓	✓	✓	✓	✓
Glucagon	20	✓	✓	✓	✓	✓
GM-CSF	21	✓	✓	✓	✓	✓
Granzyme B	22	✓	✓	✓	✓	✓
FABP3	23		✓			✓
FGF-21	24		✓			✓
GROα	25	✓	✓	✓	✓	✓
HGF	26	✓	✓	✓	✓	✓
IFNα2	27	✓	✓	✓	✓	✓
IFNγ	28	✓	✓	✓	✓	✓
IL-1α	29	✓	✓	✓	✓	✓
IL-1β	30	✓	✓	✓	✓	✓
FGF-23	31		✓			✓
HB-EGF	32		✓			✓
IL-1Ra	33	✓	✓	✓	✓	✓
IL-2	34	✓	✓	✓	✓	✓
IL-4	35	✓	✓	✓	✓	✓
IL-5	36	✓	✓	✓	✓	✓
IL-6	37	✓	✓	✓	✓	✓

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Analyte	Luminex® Bead Region	Available Panel		Instrument Compatibility		
		Fixed 80plex	Fixed or configurable 115plex	MAGPIX®	Luminex® 200™	FLEXMAP 3D® or xMAP® INTELLIFLEX
IL-7	38	✓	✓	✓	✓	✓
IL-8	39	✓	✓	✓	✓	✓
I-309	40		✓			✓
IL-11	41		✓			✓
IL-10	42	✓	✓	✓	✓	✓
IL-12 (p40)	43	✓	✓	✓	✓	✓
IL-12 (p70)	44	✓	✓	✓	✓	✓
IL-13	45	✓	✓	✓	✓	✓
IL-15	46	✓	✓	✓	✓	✓
IL-17A	47	✓	✓	✓	✓	✓
IL-18	48	✓	✓	✓	✓	✓
Eotaxin	49	✓	✓		✓	✓
LIF	50		✓			✓
IL-22	51	✓	✓	✓	✓	✓
IL-23	52	✓	✓	✓	✓	✓
IP-10	53	✓	✓	✓	✓	✓
Leptin	54	✓	✓	✓	✓	✓
MCP-1	55	✓	✓	✓	✓	✓
MIG	56	✓	✓	✓	✓	✓
MIP-1α	57	✓	✓	✓	✓	✓
FABP4	58	✓	✓		✓	✓
FLT3 Ligand	59	✓	✓		✓	✓
MCP-2	60		✓			✓
MIP-1β	61	✓	✓	✓	✓	✓
OPN	62	✓	✓	✓	✓	✓
PDGF-AA	63	✓	✓	✓	✓	✓

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		Available Panel		Instrument Compatibility		
Analyte	Luminex® Bead Region	Fixed 80plex	Fixed or configurable 115plex	MAGPIX®	Luminex® 200™	FLEXMAP 3D® or xMAP® INTELLIFLEX
PDGF-BB	64	✓	✓	✓	✓	✓
Perforin	65	✓	✓	✓	✓	✓
RANTES	66	✓	✓	✓	✓	✓
sCD40L	67	✓	✓	✓	✓	✓
GLP-1 Total	68	✓	✓		✓	✓
Granzyme A	69	✓	✓		✓	✓
I-TAC	70	✓	✓		✓	✓
MCP-4	71		✓			✓
SCF	72	✓	✓	✓	✓	✓
sFas	73	✓	✓	✓	✓	✓
sFasL	74	✓	✓	✓	✓	✓
TGFα	75	✓	✓	✓	✓	✓
TNFα	76	✓	✓	✓	✓	✓
TRAIL	77	✓	✓	✓	✓	✓
VEGF-A	78	✓	✓	✓	✓	✓
IL-3	79	✓	✓		✓	✓
IL-9	80	✓	✓		✓	✓
IL-17F	81	✓	✓		✓	✓
IL-21	82	✓	✓		✓	✓
IL-33	83	✓	✓		✓	✓
Insulin	84	✓	✓		✓	✓
M-CSF	85	✓	✓		✓	✓
MCP-3	86	✓	✓		✓	✓
MDC	87	✓	✓		✓	✓
MIP-3α	88	✓	✓		✓	✓
MMP-1	89	✓	✓		✓	✓

Analyte	Luminex® Bead Region	Available Panel		Instrument Compatibility		
		Fixed 80plex	Fixed or configurable 115plex	MAGPIX®	Luminex® 200™	FLEXMAP 3D® or xMAP® INTELLIFLEX
MMP-2	90	✓	✓		✓	✓
MMP-7	91	✓	✓		✓	✓
MIP-1ϭ	92		✓			✓
MMP-9	93	✓	✓		✓	✓
OPG	94	✓	✓		✓	✓
PlGF	95	✓	✓		✓	✓
PP	96	✓	✓		✓	✓
PYY	97	✓	✓		✓	✓
TARC	98	✓	✓		✓	✓
TNFβ	99	✓	✓		✓	✓
Troponin I	100	✓	✓		✓	✓
MIP-3β	101		✓			✓
MMP-3	102		✓			✓
MMP-10	103		✓			✓
MMP-12	104		✓			✓
Myostatin	105		✓			✓
NGF	106		✓			✓
NTproBNP	107		✓			✓
Osteocalcin	108		✓			✓
Osteonectin	109		✓			✓
SDF-1	110		✓			✓
SOST	111		✓			✓
TPO	112		✓			✓
TSLP	113		✓			✓
VEGF-C	114		✓			✓
VEGF-D	115		✓			✓

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Materials Required (Not provided)

Reagents

MAGPIX® Drive Fluid PLUS (40-50030), xMAP® Sheath Fluid PLUS (40-50021), or xMAP® Sheath Concentrate PLUS (40-50023).

Instrumentation/Materials

- Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
- Multichannel Pipettes capable of delivering 5 µL to 50 µL, or 25 µL to 200 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Rubber Bands
- Aluminum Foil
- Absorbent Pads
- Laboratory Vortex Mixer
- Sonicator (Branson Ultrasonic Cleaner Model No. B200 or equivalent)
- MultiscreenHST BV 96-well filter plate (Catalogue No. MSBVN1210 or equivalent)
- Titer Plate Shaker (VWR® Microplate Shaker Catalogue No. 12620-926 or equivalent)
- Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, Cat. Nos. 40-092AB, 40-094, 40-095, 40-096, 40-097 or equivalent) or Handheld Magnetic Separation Block (Catalogue No. 40-285 or equivalent).
- Luminex® 200™, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX with INTELLIFLEX software by Luminex® Corporation.

  Note: See Table on pages 5-9 for instrument requirements based on analyte selection.

Safety Precautions

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide or ProClin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and ProClin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Symbol Definitions

Ingredient	Catalogue No.	Label	
PLEXpedition Standard 1 (10X) PLEXpedition Standard 2 (10X) PLEXpedition Standard 3 (10X) PLEXpedition Standard 4 (10X) PLEXpedition Standard 5 (10X) PLEXpedition Standard 6 (10X) PLEXpedition Standard 7 (10X)	HPLX1-9010-01 HPLX1-9010-02 HPLX1-9010-03 HPLX1-9010-04 HPLX1-9010-05 HPLX1-9010-06 HPLX1-9010-07		**Danger.** Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.
PLEXpedition Detection Antibody PLEXpedition Custom Premixed Detection Antibody	HPLX1-2010		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Streptavidin-Phycoerythrin	HPLX-SAPE		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
10X Wash Buffer	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Technical Guidelines

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25 °C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20 °C for 1 month and at ≤ -80 °C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8 °C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.

- When reading the assay on Luminex® 200™, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.
- For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid PLUS in each well and 75 µL should be aspirated.
- For xMAP® INTELLIFLEX, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.
- The PLEXpedition Screening Panel is designed for screening in Assay Buffer. If running a matrix curve is desired, it is recommended to also run the standard curve in Assay Buffer, in parallel, as a control. If using a matrix (not included in the kit), please see the recommendations below for common sample types:
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, consult with Technical Support for potential serum matrix options and their respective analyte compatibility. When sample dilution is required, use the Assay Buffer provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

Sample Collection and Storage

Analytes may vary in abundance. Neat samples are recommended for initial testing with PLEXpedition Screening Panel. Further dilution by the end user may improve performance for certain sample types and/or analytes. When sample dilution is required, use Assay Buffer as the diluent. Provided below are preparation instructions for common sample types.

Preparation of Serum Samples

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 x *g*. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and prepare using one of the following methods:
- Use a filter plate with pre-filter (recommended method): Stack the filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for 10 minutes at ≥ 1,100 x *g*.

- Use a microcentrifuge: centrifuge samples at > 13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

Preparation of Plasma Samples

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and prepare using one of the following methods:
- Use a filter plate with pre-filter (recommended method): Stack the filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for 10 minutes at ≥ 1,100 x *g*.
- Use a microcentrifuge: centrifuge samples at > 13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.

Preparation of Tissue Culture Supernatant

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

Note:

- A maximum of 25 µL per well of neat or diluted serum, plasma, or other biofluid can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. DO NOT STORE SAMPLES IN GLASS.
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Preparation of Reagents for Immunoassay

Preparation of Premixed Antibody-Immobilized Beads

Sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.

Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.

Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

Preparation of MILLIPLEX® PLEXpedition Screening Panel Standards

1. Prior to use, reconstitute each PLEXpedition Standard (10X) with 25 µL deionized water.

   Note: Briefly centrifuge the vial before opening the cap for reconstitution. Invert the vial several times to mix. Gently vortex each vial for 10 seconds then centrifuge briefly. Allow the vial to sit for 5-10 minutes.

2. To prepare 1X standard, transfer 20 µL of each reconstituted standard to a polypropylene microfuge tube and bring the final volume up to 200 µL with Assay Buffer.

   For example, if 7 PLEXpedition standards are provided, add 20 µL from each reconstituted vial and bring the total volume to 200 µL with 60 µL of Assay Buffer. This will be used as "Standard 7"; the unused portion may be stored at ≤ -20 °C for up to one month.

   For example, if 3 PLEXpedition standards are provided, add 20 µL from each reconstituted vial and bring the total volume to 200 µL with 140 µL of Assay Buffer. This will be used as "Standard 7"; the unused portion may be stored at ≤ -20 °C for up to one month.

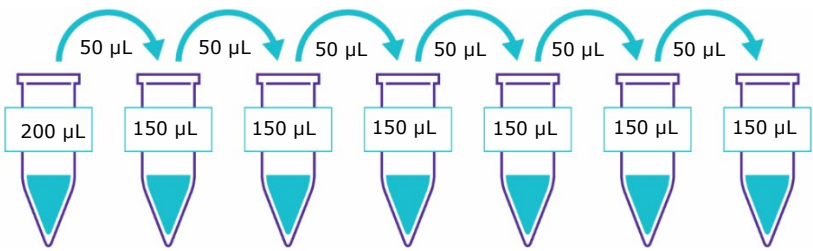
Preparation of Working Standards

Label 6 polypropylene microfuge tubes Standard 1 through Standard 6. Add 150 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 50 µL of the reconstituted standard to the Standard 6 tube, mix well and transfer 50 µL of Standard 6 to the Standard 5 tube, mix well and transfer 50 µL of Standard 5 to the Standard 4 tube, mix well and transfer 50 µL of Standard 4 to the Standard 3 tube, mix well and transfer 50 µL of Standard 3 to the Standard 2 tube, mix well and transfer 50 µL of Standard 2 to the Standard 1 tube and mix well. The 0 standard (Background) will be Assay Buffer.

Standard No.	Add Assay Buffer (µL)	Add 10X Standard (volume)
Standard 7	Bring total volume to 200 µL	20 µL per vial

Standard No.	Add Assay Buffer (µL)	Add Standard (volume)
Standard 6	150 µL	50 µL of Standard 7
Standard 5	150 µL	50 µL of Standard 6
Standard 4	150 µL	50 µL of Standard 5
Standard 3	150 µL	50 µL of Standard 4
Standard 2	150 µL	50 µL of Standard 3
Standard 1	150 µL	50 µL of Standard 2

Preparation of Standards



Combined Standard 7 | Standard 6 | Standard 5 | Standard 4 | Standard 3 | Standard 2 | Standard 1

NOTE: Refer to product insert(s) for analyte concentrations.

Immunoassay Procedure

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25 °C) before use in the assay.
- Diagram the placement of Standards 0 (Background), Standard 1 through 7, and Samples on Well Map Worksheet in a vertical configuration. It is recommended to run the assay in duplicate.

  Note: Most instruments will read the 96-well plate vertically by default.

1. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25 °C).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard into the appropriate wells. Assay Buffer should be used for 0 standard (Background).
4. Add 25 µL of Assay Buffer to the background, standard, and sample wells.
5. Add 25 µL of Sample (neat or diluted) into the appropriate wells.
6. Vortex Mixing Bottle and add 25 µL of the Mixed or Premixed Beads to each well.

   Note: During addition of Beads, shake bead bottle intermittently to avoid settling.
7. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8 °C. If a same day assay is preferred, please contact Technical Support for selected analyte compatibility with a 2 hour incubation at room temperature (20-25 °C) option.

Add 200 µL Wash Buffer per well

Shake 10 min, RT

Decant

- Add 25 µL Standard to appropriate wells.
- Add 25 µL Assay Buffer to background wells.
- Add 25 µL Assay Buffer to background, standards, and sample wells.
- Add 25 µL neat or diluted Samples to sample wells.
- Add 25 µL Beads to each well.

Incubate overnight (16-18 hours) at 2-8 °C

8. Gently remove well contents and wash plate 3 times following instructions listed in the PLATE WASHING section.
9. Add 25 µL of Detection Antibodies into each well.
10. Note: Allow the Detection Antibodies to warm to room temperature prior to addition.
11. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25 °C).
12. Gently remove well contents and wash plate 3 times following instructions listed in the PLATE WASHING section.
13. Add 25 µL Streptavidin-Phycoerythrin to each well.
14. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
15. Gently remove well contents and wash plate 3 times following instructions listed in the PLATE WASHING section.
16. Add 150 µL of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
17. Run plate on Luminex® 200™, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX with INTELLIFLEX Software. (See page 7-10 to confirm instrument compatibility with analyte selection.)
18. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples.

    Note: No sample dilution is required for this assay. If samples were diluted, final sample concentrations should be multiplied by the dilution factor.

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 Hour at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate 30 minutes at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 150 µL Sheath Fluid PLUS or Drive Fluid PLUS per well

Read on Luminex® instrument (100 µL, 50 beads per bead set)

Plate Washing

Magnetic plate washer is the recommended method of washing to limit bead count issues in sample wells.

Solid Plate

- Handheld Magnetic Separator Block (Catalogue No. 40-285)

  Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

- Magnetic plate washer (Catalogue Nos. 40-094, 40-095, 40-096 and 40-097)

  Please refer to specific automatic plate washer manual for appropriate equipment settings. Please note that after the final aspiration, there will be approximately 25 µL of residual wash buffer in each well. This is expected when using the BioTek® plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek® 405 LS or 405 TS, please refer to the manufacturer's recommendations for programming instructions.

Equipment Settings

Luminex® 200™, FLEXMAP 3D®, MAGPIX® with xPONENT® software and xMAP® INTELLIFLEX with INTELLIFLEX software:

These specifications are for the above listed instruments and software. Luminex® instruments with other software (for example MasterPlex®, StarStation, LiquiChip, Bio-Plex® Manager™, LABScan™100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex® magnetic beads.

For magnetic bead assays, each instrument must be calibrated, and performance verified with the indicated calibration and verification kits.

Instrument	Calibration Kit	Verification Kit
Luminex® 200™	xPONENT® 3.1 compatible Calibration Kit (Catalogue No. LX2R-CAL-K25)	Performance Verification Kit (Catalogue No. LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Catalogue No. F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (Catalogue No. F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (Catalogue No. IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (Catalogue No. IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (Catalogue No. MPX-CAL-K25)	MAGPIX® Performance Verification Kit (Catalogue No. MPX-PVER-K25)

Note: When setting up a Protocol using the xPONENT® software, you must select MagPlex® as the Bead Type in the Acquisition settings.

Note: These assays cannot be run on any instruments using Luminex® IS 2.3 or Luminex® 1.7 software.

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Catalogue No. MAG-PLATE, if additional plates are required for this purpose.

Events	50, per bead
Sample Size	100 µL
Gate Settings	8,000 to 15,000
Reporter Gain	Default (low PMT)
Time Out	60 seconds
Bead Set	See table of configurable beads on pages 5-9

Assay Characteristics

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel, with the following exceptions:

GIP and ENA-78 have low cross-reactivity with IL-23 (≤15%) and GROα (≤5%) recombinant proteins, respectively.

IL-12 (p40) has moderate, but expected, cross-reactivity with the recombinant proteins for IL-12 (p70) and IL-23 which both contain the p40 subunit.

MMP-3 may exhibit significant cross-reactivity with MMP-10 recombinant proteins due to high sequence homology, however, analysis demonstrated independent and differentiated measurement of MMP-3 and MMP-10 in biological samples.

Please contact Technical Support for additional information.

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the % CV's across two different concentrations of analytes across 4 different assays

Intra-assay precision was less than 15% for all analytes and inter-assay CV is less than 20% for all analytes except PDGF-AA which is less than 25%.

Troubleshooting

Problem	Probable Cause	Solution
Insufficient bead count	Plate washer aspirate height set too low	Adjust aspiration height according to manufacturers' instructions.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flushes, back flushes, and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex® 200™, adjust probe height to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height to the kit solid plate using 1 alignment disc. For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid PLUS in each well and 75 µL should be aspirated.
		When reading the assay on xMAP® INTELLIFLEX, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipettes without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross-reacting components (for example, interleukin modified tissue culture medium). When testing a potential matrix, it is recommended to also run standard curves in assay buffer as a control.
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex® instrument not calibrated correctly or recently	Calibrate Luminex® instrument based on manufacturer's instructions, at least once a week or if temperature has changed by >3 °C.
	Gate settings not adjusted correctly	Some Luminex® instruments (for example Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with Sheath Fluid PLUS or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.

Problem	Probable Cause	Solution
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol.
	Incubations done at inappropriate temperatures, timings, or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex® instruments (for example, Bio-Plex®) default target setting for RP1 calibrator is set at high PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Alternatively, adding a matrix to suppress the standard curves and background may improve detectability for some analytes. Check with technical support for appropriate protocol modifications or potential matrix options for curve suppression.
	Samples contain analyte oncentrations higher than highest standard point	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve	See above.

Problem	Probable Cause	Solution
High variation in samples and/or standards	Multichannel pipette may not be calibrated	Calibrate pipettes.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate.

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	Standard 4	Sample 1									
B	Standard 0 (Background)	Standard 4	Sample 1									
C	Standard 1	Standard 5	Sample 2									
D	Standard 1	Standard 5	Sample 2									
E	Standard 2	Standard 6	Etc.									
F	Standard 2	Standard 6	Etc.									
G	Standard 3	Standard 7										
H	Standard 3	Standard 7										

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