Magnetic Luminex® Performance Assay

Human Adhesion Molecule Multiplex Kit

Catalog Number LKTM007

For the simultaneous quantitative determination of multiple human cell adhesion molecules in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Adhesion molecules play a critical role during inflammatory responses by mediating the interactions of leukocytes to endothelial cells and, subsequently, their migration into perivascular tissues. The levels of adhesion molecules such as intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, endothelial (E)-Selectin, and platelet (P)-Selectin are generally low in normal tissues but increase during an inflammatory response. Soluble forms are detected in biological fluids, and their concentrations may reflect their cellular expression levels.

This kit can be used to simultaneously assess the levels of the four adhesion molecules listed below in a single sample. For ease of use, the adhesion molecule microparticles are pre-mixed in one vial and the biotinylated detection antibodies are pre-mixed as well.

Analyte	Bead Region
ICAM-1	18
E-Selectin	39
P-Selectin	29
VCAM-1	35

PRINCIPLE OF THE ASSAY

Magnetic Luminex® Performance Assay multiplex kits are designed for use with the Luminex® MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex® 100/200™ or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique bead region. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex® MAGPIX® Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the beads. One LED excites the dyes inside each bead to identify the region and the second LED excites the PE to measure the amount of analyte bound to the bead. A sample from each well is imaged with a CCD camera with a set of filter to differentiate excitation levels.

Analysis with the Luminex® 100/200™ or Bio-Rad Bio-Plex uses one laser to excite the dyes inside each bead to identifying the bead region and the second laser to excite the PE to measure the amount of analyte bound to the bead. All excitation emitted as each bead passes through the flow cell is then analyzed to differentiate excitation levels using a Photomultiplier Tube (PMT) and an Avalanche Photodiode.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until these factors have been tested in the Magnetic Luminex® Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex® Performance Assays afford the user the benefit of multi-analyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions, therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

PRECAUTIONS

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Human Adhesion Panel Standard Cocktail	893808	2 vials of recombinant human adhesion molecules in a buffered protein base with preservatives; lyophilized.	Use a fresh standard for each assay. Discard after use.
Calibrator Diluent RD6-55	895338	21 mL of a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	May be stored for up to 1 month at 2-8 °C.* *Once diluted, 1X solutions must be discarded. Use fresh diluents for each assay.*
Human Adhesion Panel Microparticle Cocktail	898611	0.60 mL of a 20X concentrated human adhesion molecule microparticle cocktail with preservatives.	
Streptavidin-PE	892525	0.07 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Human Adhesion Panel Biotin Ab Cocktail	893807	0.60 mL of a 10X concentrated human adhesion molecule biotin antibody cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Biotin Antibody Diluent 2	895832	5.5 mL of a buffered protein base with preservative.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Plate Sealers	640445	4 adhesive foil strips.	
Standard Value Card	749922	1 sticker listing the reconstitution volume and working concentrations for this lot of standard.	

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Luminex® MAGPIX®, Luminex® 100/200™, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm.
- Microcentrifuge.
- 15 mL amber conical tubes (Greiner Bio-One, Catalog # 188280 or equivalent) for the dilution of microparticles, biotin antibody, and Streptavidin-PE.
- **Polypropylene** test tubes for dilution of standards and samples.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 30 µL of sample + 270 µL of Calibrator Diluent RD6-55 (diluted 1:2)*. Mix thoroughly.

*See Reagent Preparation section.

REAGENT PREPARATION

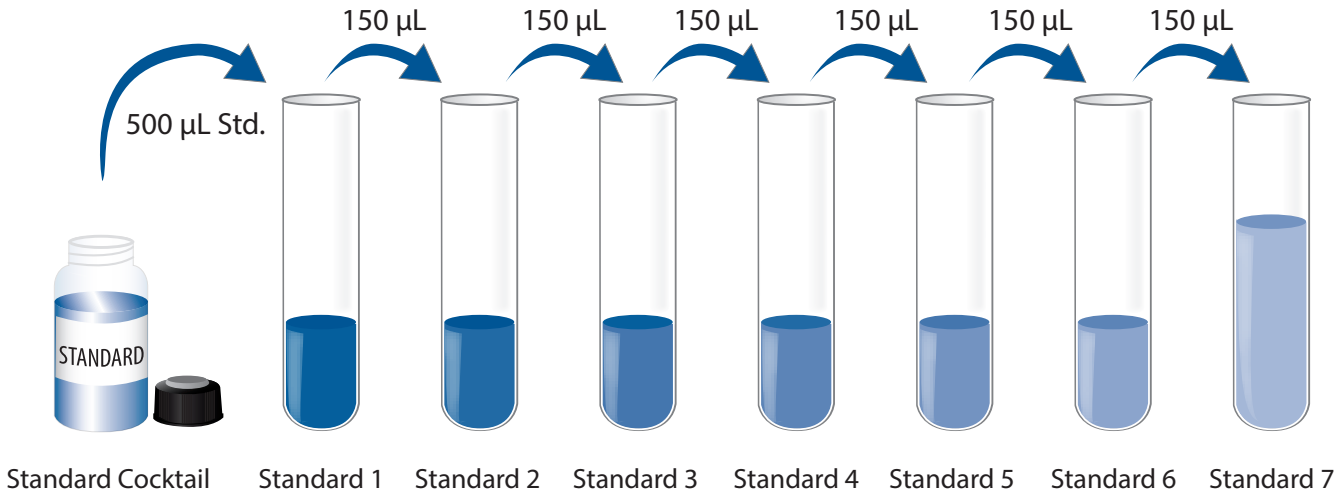
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer. If assaying a partial plate, prepare only as much Wash Buffer as needed.

Calibrator Diluent RD6-55 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD6-55 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-55 (diluted 1:2). If assaying a partial plate, prepare only as much Calibrator Diluent RD6-55 (diluted 1:2) as needed.

Standard - Reconstitute the Standard Cocktail with Calibrator Diluent RD6-55 (diluted 1:2). Refer to the Standard Value Card for the reconstitution volume and assigned values. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of the reconstituted Standard into the Standard 1 tube. Pipette 300 µL of Calibrator Diluent RD6-55 (diluted 1:2) into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD6-55 (diluted 1:2) serves as the blank.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in a polypropylene amber tube or a polypropylene tube wrapped with aluminum foil.

Number of Wells Used	Microparticle Cocktail	+	Assay Diluent RD1W
96	500 µL	+	10.0 mL
72	375 µL	+	7.5 mL
48	250 µL	+	5.0 mL
24	125 µL	+	2.5 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin Antibody vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin Antibody Cocktail in Biotin Antibody Diluent 2. Mix gently.

Number of Wells Used	Biotin Antibody Cocktail	+	Biotin Antibody Diluent 2
96	500 µL	+	5.0 mL
72	375 µL	+	3.75 mL
48	250 µL	+	2.5 mL
24	125 µL	+	1.25 mL

Note: *Diluted biotin antibody cannot be stored. Prepare within 30 minutes of use.*

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Streptavidin-PE in Wash Buffer.

Number of Wells Used	Streptavidin-PE	+	Wash Buffer
96	55.0 µL	+	5.50 mL
72	42.0 µL	+	4.10 mL
48	28.0 µL	+	2.75 mL
24	14.0 µL	+	1.35 mL

INSTRUMENT SETTINGS

Luminex® MAGPIX® analyzer:

a) Assign the microparticle region for each analyte being measured (see page 1)

b) 50 events/bead

c) Sample size: 50 µL

d) Collect Median Fluorescence Intensity (MFI)

Luminex® 100/200™ and Bio-Rad Bio-Plex analyzers:

Note: *Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

a) Assign the bead region for each analyte being measured (see page 1)

b) 50 events/bead

c) Minimum events: 0

d) Flow rate: 60 µL/minute (fast)

e) Sample size: 50 µL

f) Doublet Discriminator Gates

i. Luminex 100/200TM set at 8000 and 16,500

ii. Bio-Rad Bio-Plex analyzer set at 8000 and 23,000

g) Reporter Gain Setting should remain on Default for accurate data analysis.

h) Collect MFI

Note: *The CAL2 setting for the Bio-Rad Bio-Plex analyzer should be set at the low RP1 target value.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 100 µL of standard, control, or sample* per well.
3. Resuspend the diluted microparticle cocktail by inversion or vortexing. Add 100 µL of the microparticle cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 1 minute before removing the liquid, filling each well with Wash Buffer (100 µL) and allow 1 minute before removing the liquid again. Complete removal of liquid is essential for good performance. **Note:** *Do NOT blot; this may cause a loss of microparticles.* Perform the wash procedure three times.

   Note: *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*
5. Add 50 µL of diluted Biotin Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex® or Bio-Rad analyzer.
    Note: *Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker set at 800* ± 50 rpm.

*Samples may require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

❶ Prepare all reagents as instructed.

↓

❷ Add 100 µL of standard, control, or sample* to each well.

↓

❸ Add 100 µL of microparticle cocktail to each well.
Incubate for 2 hours at RT on a shaker at 800 rpm.

↓

❹ Wash by removing the liquid from each well, filling with 100 µL Wash Buffer, and removing the liquid again.
Perform the wash 3 times.

↓

❺ Add 50 µL of diluted Biotin-Antibody Cocktail to each well.
Cover and incubate for 1 hour at RT on the shaker at 800 rpm.

↓

❻ Repeat the wash as in step 4.

↓

❼ Add 50 µL of diluted Streptavidin-PE to each well.
Incubate for 30 minutes at RT on the shaker at 800 rpm.

↓

❽ Repeat the wash as in step 4.

↓

❾ Add 100 µL of Wash Buffer to each well.
Incubate for 2 minutes at RT on the shaker at 800 rpm.

↓

❿ Read within 90 minutes using a Luminex® or Bio-Rad analyzer
Note: *Resuspend microparticles immediately prior to reading.*

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Use the standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

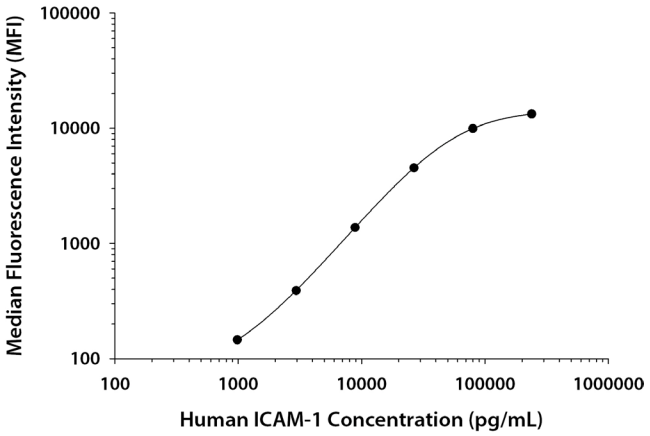
Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

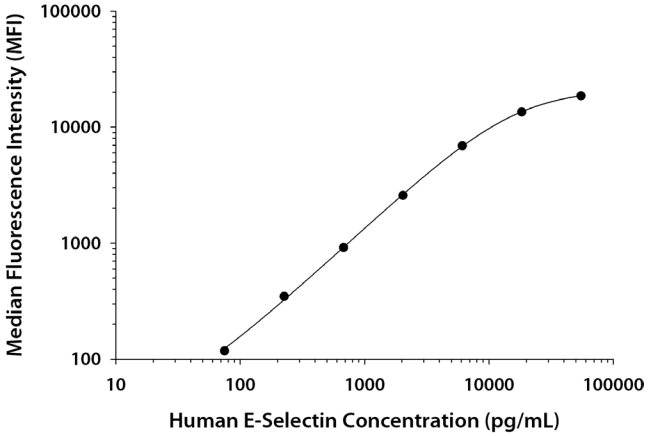
These standard curves are provided only for demonstration. Standard curves must be generated each time an assay is run, utilizing values from the Standard Value Card.

ICAM-1



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	78 81	80	—
Standard 1	239,400	13,355 13,394	13,374	13,294
Standard 2	79,800	9966 10,064	10,015	9935
Standard 3	26,600	4454 4765	4610	4530
Standard 4	8867	1430 1485	1458	1378
Standard 5	2956	464 478	471	391
Standard 6	985	207 245	226	146

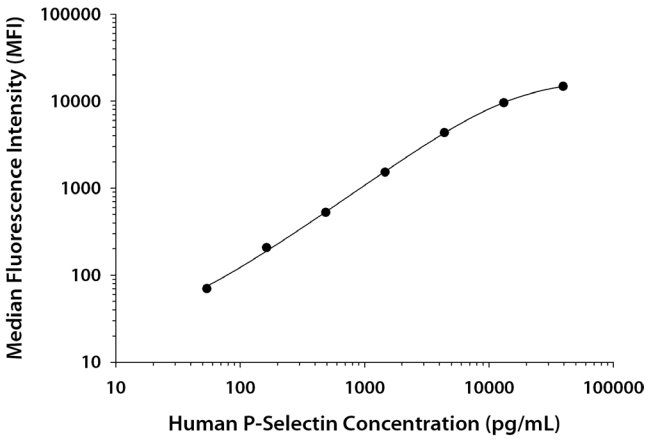
E-Selectin



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	14 14	14	—
Standard 1	55,000	18,554 18,798	18,676	18,662
Standard 2	18,333	13,432 13,640	13,536	13,522
Standard 3	6111	6763 7086	6924	6910
Standard 4	2037	2551 2642	2596	2582
Standard 5	679	920 946	933	919
Standard 6	226	351 374	362	348
Standard 7	75	132 133	132	118

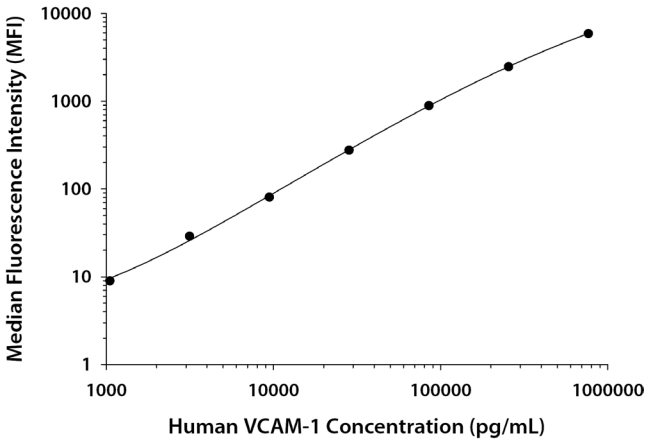
TYPICAL DATA *CONTINUED*

P-Selectin



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	54 54	54	—
Standard 1	39,500	14,749 14,932	14,840	14,786
Standard 2	13,167	9604 9708	9656	9602
Standard 3	4389	4306 4494	4400	4346
Standard 4	1463	1564 1599	1582	1528
Standard 5	488	574 589	582	528
Standard 6	163	252 273	262	208
Standard 7	54	123 125	124	70

VCAM-1



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	9 9	9	—
Standard 1	765,400	5861 5950	5906	5897
Standard 2	255,133	2472 2489	2480	2471
Standard 3	85,044	885 911	898	889
Standard 4	28,348	280 291	286	277
Standard 5	9449	89 92	90	81
Standard 6	3150	36 40	38	29
Standard 7	1050	17 18	18	9

CALIBRATION

This assay is calibrated against highly purified recombinant human adhesion molecules produced at R&D Systems®.

PERFORMANCE CHARACTERISTICS

Data obtained with polystyrene and magnetic beads were equivalent.

SENSITIVITY

Twenty-nine assays were run and the minimum detectable dose (MDD) was determined by adding two standard deviations to the MFI of twenty zero standard replicates and calculating the corresponding concentration.

Analyte	Mean (pg/mL)	Range (pg/mL)
ICAM-1	130	64-303
E-Selectin	2.1	0.9-7.4

Analyte	Mean (pg/mL)	Range (pg/mL)
P-Selectin	6.4	3.0-12.2
VCAM-1	252	122-529

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in eighty-seven separate assays to assess inter-assay precision.

ICAM-1

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	87	87	87
Mean (pg/mL)	5120	42,130	1704	5622	53,471	1921
Standard deviation	297	1498	141	813	8683	324
CV (%)	5.8	3.6	8.3	14.5	16.2	16.9

E-Selectin

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	87	87	87
Mean (pg/mL)	996	8693	611	948	8491	561
Standard deviation	28	509	20	113	798	65
CV (%)	2.8	5.9	3.3	11.9	9.4	11.6

P-Selectin

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	87	87	87
Mean (pg/mL)	2168	17,455	512	1999	18,652	447
Standard deviation	100	1029	24	190	1640	54
CV (%)	4.6	5.9	4.7	9.5	8.8	12.1

VCAM-1

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	87	87	87
Mean (pg/mL)	25,870	223,400	8608	23,543	221,030	8283
Standard deviation	1344	6754	347	2213	14,061	956
CV (%)	5.2	3.0	4.0	9.4	6.4	11.5

RECOVERY

The recovery of each analyte spiked to levels throughout the range of the assay in various matrices was evaluated.

ICAM-1

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	89	79-101%
Serum (n=8)	105	96-119%
EDTA plasma (n=8)	99	82-122%
Heparin plasma (n=8)	98	84-120%

E-Selectin

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	95	91-101%
Serum (n=8)	97	88-106%
EDTA plasma (n=8)	93	84-109%
Heparin plasma (n=8)	92	88-102%

P-Selectin

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	95	91-99%
Serum (n=8)	99	86-116%
EDTA plasma (n=8)	91	86-109%
Heparin plasma (n=8)	94	79-112%

VCAM-1

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	90	82-106%
Serum (n=8)	97	86-124%
EDTA plasma (n=8)	92	82-115%
Heparin plasma (n=8)	92	81-117%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of each analyte were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Serum and plasma samples were diluted prior to assay as directed in the Sample Preparation section.

ICAM-1		Cell culture supernates	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	102	107	105	105
	Range (%)	89-118	92-117	91-113	94-120
1:4	Average % of Expected	106	107	106	106
	Range (%)	103-109	84-122	90-122	92-129
1:8	Average % of Expected	113	102	95	98
	Range (%)	109-118	80-118	74-108	85-114

E-Selectin		Cell culture supernates	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	101	96	98	100
	Range (%)	90-114	86-107	89-107	85-112
1:4	Average % of Expected	102	101	100	101
	Range (%)	92-111	88-113	88-114	92-111
1:8	Average % of Expected	105	107	105	102
	Range (%)	97-114	94-116	88-119	86-122

P-Selectin		Cell culture supernates	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	99	104	102	103
	Range (%)	95-103	96-111	90-112	97-110
1:4	Average % of Expected	96	106	106	108
	Range (%)	78-108	88-117	89-115	100-116
1:8	Average % of Expected	111	113	109	112
	Range (%)	107-114	100-124	92-125	98-124

VCAM-1		Cell culture supernates	Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	108	97	92	97
	Range (%)	96-116	83-108	83-105	87-104
1:4	Average % of Expected	117	96	89	93
	Range (%)	113-122	82-107	81-99	85-108
1:8	Average % of Expected	108	99	91	92
	Range (%)	105-111	82-111	75-109	81-100

SPECIFICITY

This assay recognizes natural and recombinant human ICAM-1, E-Selectin, P-Selectin, and VCAM-1.

Cross-reactivity - The factors listed below were prepared at 500 ng/mL in calibrator diluent, assayed, and measured less than 0.8% cross-reactivity.

Recombinant human:

ALCAM
BCAM
Cadherin-8
Cadherin-11
CEACAM-1
CHL-1
CNTN-2
CNTN-4
Contactin-1
DC-SIGN R
Desmoglein-1
Desmoglein-2
DNAM-1
E-Cadherin
E-Calectin
Endocan
EpCAM
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7
Galectin-8
ICAM-2
ICAM-3
ICAM-5
JAM-1
JAM-2
JAM-3
LOX-1
L-Selectin
M-Cadherin
MCAM
N-Cadherin
NCAM-L1
NrCAM
P-Cadherin
PECAM
Siglec-2
Siglec-3
Siglec-5
Siglec-6
Siglec-7
Siglec-9
Siglec-10
TROP-2
VE-Cadherin

Recombinant mouse:

ALCAM
CHL-1
DCC
E-Cadherin
Endocan
E-Selectin
Galectin-1
Galectin-3
Galectin-7
ICAM-1
ICAM-2
ICAM-5
JAM-1
JAM-2
JAM-5
LOX-1
L-Selectin
MAdCAM-1
P-Cadherin
Siglec-2
Siglec-F
VCAM-1
VE-Cadherin

Recombinant rat:

E-Selectin
ICAM-1
L-Selectin
MAG

ICAM-1 Multiplex Partners:

E-Selectin
P-Selectin
VCAM-1

E-Selectin Multiplex Partners:

ICAM-1
P-Selectin
VCAM-1

P-Selectin Multiplex Partners:

ICAM-1
E-Selectin
VCAM-1

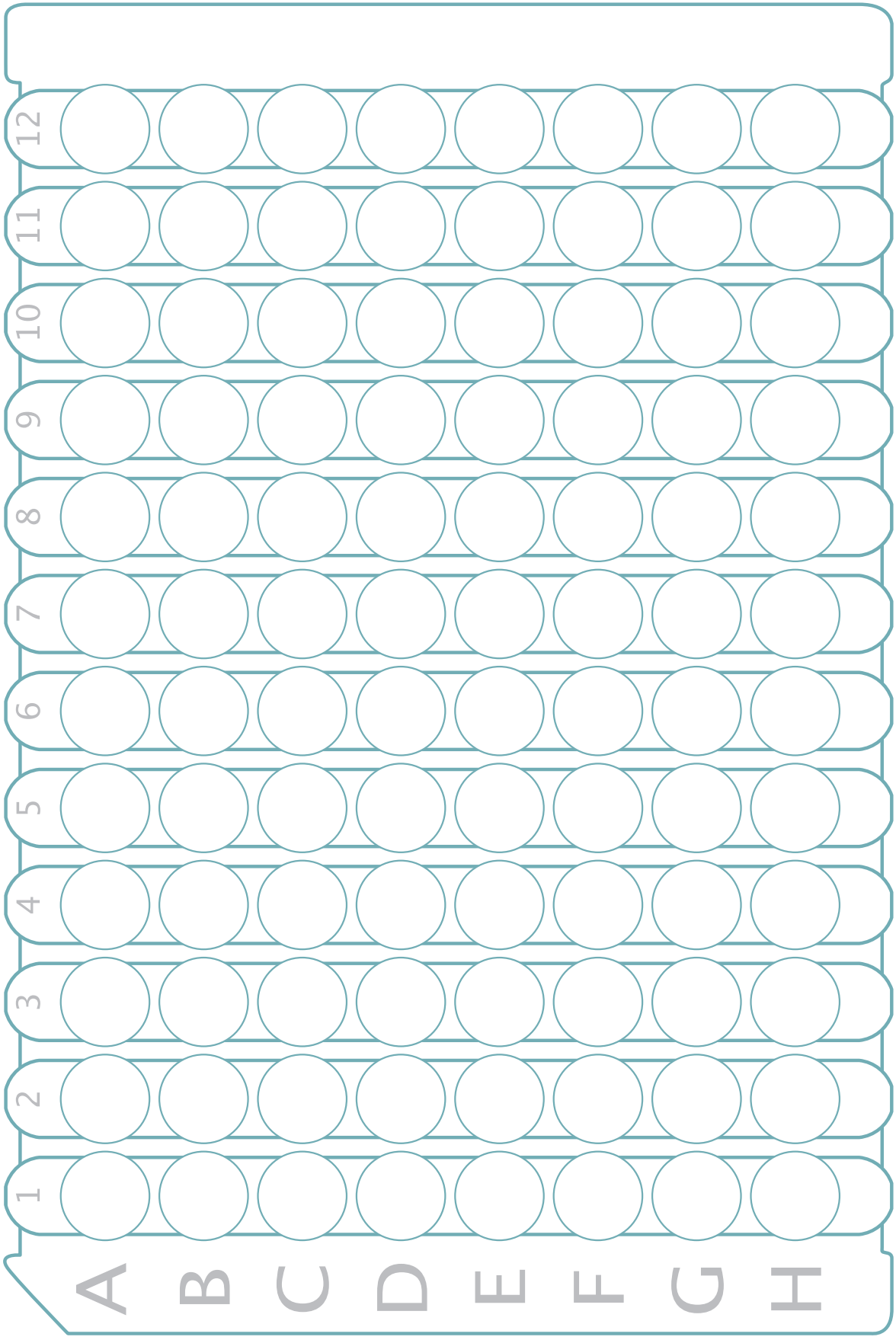
VCAM-1 Multiplex Partners:

ICAM-1
E-Selectin
P-Selectin

Interference - Preparations of the factors listed above were prepared at 500 ng/mL in a mid-range recombinant human adhesion molecule control and assayed for interference. None of the factors interfered in the assay.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES