Quantikine™ ELISA

Human EMMPRIN/CD147 Immunoassay

Catalog Number DEMP00

For the quantitative determination of human Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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

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INTRODUCTION

Extracellular Matrix Metalloproteinase Inducer (EMMPRIN), also known as CD147, basigin, leukocyte activation antigen M6, collagenase stimulatory factor, tumor cell-derived stimulatory factor, and OK blood antigen, is a 35-65 kDa, variably glycosylated, type I transmembrane glycoprotein that belongs to the immunoglobulin superfamily of receptors (1). Human EMMPRIN is synthesized as a 269 amino acid (aa) precursor that contains a 24 aa signal sequence, a 183 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 41 aa intracellular domain. The ECD contains one C2-type and one V-type Ig-like domain. A 385 aa splice variant exists that contains an N-terminal IgCAM domain and is found only in the retina (2). mRNA transcripts, but not protein, have been reported for additional 432, 388, 205, 176, and 174 aa variants.

EMMPRIN is expressed in areas of tissue remodeling, including tumors, endometrium, placenta, skin, and regions undergoing angiogenesis (3-8). It is also expressed in cells with high metabolic activity, such as lymphoblasts, macrophages, and tumor cells (4, 9). On cells with elevated metabolic rates, EMMPRIN is often co-expressed with the amino acid transporter CD98h (10). EMMPRIN also interacts with caveolin-1 via its C2-like domain, and this reduces the level of EMMPRIN glycosylation and subsequent EMMPRIN multimerization and activity (11). EMMPRIN's transmembrane sequence contains a Glu and a Pro which are important for intracellular interactions with cyclophilins (CyP) (12-14). CyPA (cyclophilin A) and CyP60 interactions with the transmembrane segment promote leukocyte inflammatory chemotaxis and surface expression of EMMPRIN, respectively (13-14). An active 22 kDa fragment can be shed from tumor cells by MMP-14/MT1-MMP (3). Tumor cells can also release active, full-length EMMPRIN in microvesicles (15-16). Functionally, EMMPRIN is known to induce urokinase-type plasminogen activator (uPA), VEGF, hyaluronan, and multiple MMPs (3-4, 6-8). Human EMMPRIN (269 aa) shows 58% aa sequence identity with mouse and rat EMMPRIN. It also shows 25% and 38% aa sequence identity with the related proteins embigin and neuroplastin (SDR-1), respectively (17).

The Quantikine™ Human EMMPRIN/CD147 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human EMMPRIN in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains NS0-expressed recombinant human EMMPRIN and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human EMMPRIN showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally ocurring human EMMPRIN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human EMMPRIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EMMPRIN present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human EMMPRIN is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of EMMPRIN bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 generate values higher than the highest standard, 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 all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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/ RECONSTITUTED MATERIAL
Human EMMPRIN Microplate	892344	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human EMMPRIN.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human EMMPRIN Conjugate	892422	21 mL of a polyclonal antibody specific for human EMMPRIN conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human EMMPRIN Standard	892423	Recombinant human EMMPRIN in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain crystals. Mix well before and during use.*	
Calibrator Diluent RD6-8	895181	21 mL of buffered animal serum with preservatives. *For serum/plasma samples.*	
Calibrator Diluent RD6-14	895220	21 mL of a buffered protein base with preservatives. *For cell culture supernates/ saliva/urine/human milk samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human EMMPRIN Controls (optional; R&D Systems®, Catalog # QC80)

PRECAUTIONS

Calibrator Diluent RD6-8 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

EMMPRIN is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

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

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

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

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 centrifugation 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.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-8.

Urine samples require at least a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-14.

Human milk samples require at least a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD6-14.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

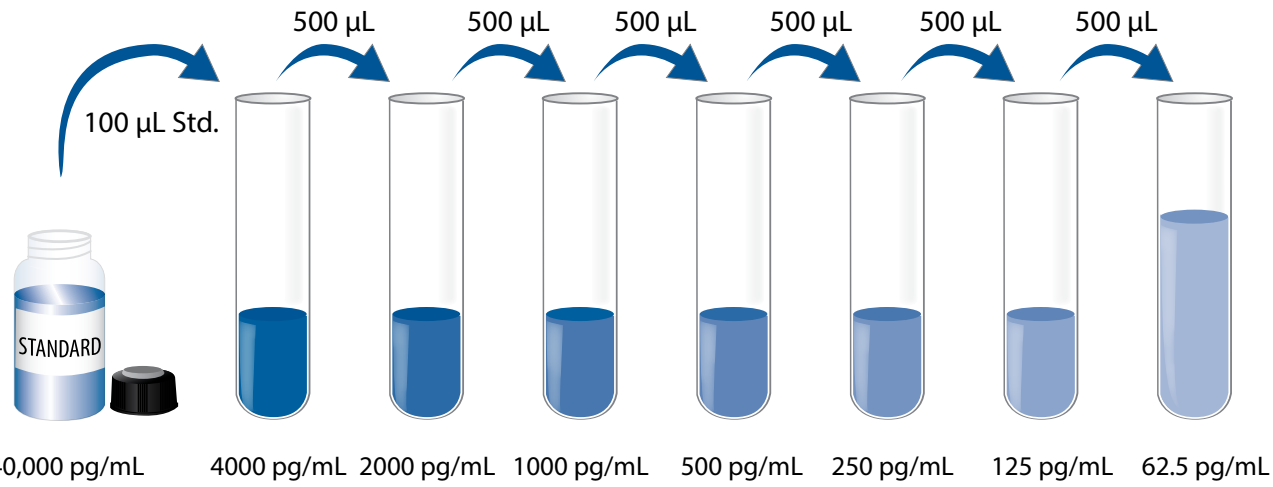
Note: *High concentrations of EMMPRIN are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human EMMPRIN Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human EMMPRIN Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD6-8 (*for serum and plasma samples*) or Calibrator Diluent RD6-14 (*for cell culture supernate, saliva, urine, and human milk samples*) into the 4000 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

Note: *High concentrations of EMMPRIN are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1X to each well. *Assay Diluent RD1X may contain crystals. Warm to room temperature. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human EMMPRIN Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

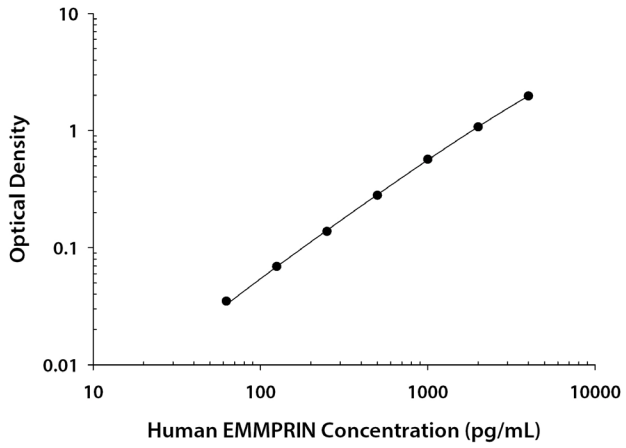
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human EMMPRIN concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

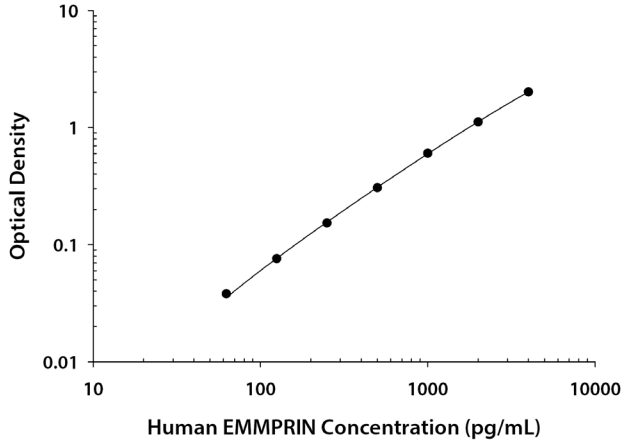
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
62.5	0.044 0.044	0.044	0.035
125	0.076 0.080	0.078	0.069
250	0.146 0.148	0.147	0.138
500	0.287 0.290	0.289	0.280
1000	0.571 0.583	0.577	0.568
2000	1.074 1.092	1.083	1.074
4000	1.959 2.002	1.981	1.972

CELL CULTURE SUPERNATES/SALIVA/URINE/HUMAN MILK ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.010	0.010	—
62.5	0.047 0.049	0.048	0.038
125	0.084 0.088	0.086	0.076
250	0.161 0.164	0.163	0.153
500	0.309 0.322	0.316	0.306
1000	0.605 0.623	0.614	0.604
2000	1.108 1.147	1.128	1.118
4000	1.995 2.058	2.027	2.017

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA/URINE/HUMAN MILK ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	448	1257	2412	396	1183	2363
Standard deviation	20.1	43.0	120	21.1	70.5	120
CV (%)	4.5	3.4	5.0	5.3	6.0	5.1

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	431	1208	2341	421	1240	2474
Standard deviation	15.3	46.9	109	23.7	54.9	145
CV (%)	3.5	3.9	4.7	5.6	4.4	5.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	91-111%
Serum* (n=4)	100	90-111%
EDTA plasma* (n=4)	98	90-110%
Heparin plasma* (n=4)	96	85-104%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human EMMPRIN were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva* (n=4)	Urine* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	105	100	94	102	103	102	104
	Range (%)	104-107	97-102	90-101	99-104	101-106	99-104	101-108
1:4	Average % of Expected	104	101	94	98	102	102	107
	Range (%)	99-110	95-105	92-97	92-102	100-104	97-105	105-109
1:8	Average % of Expected	104	102	94	97	102	101	106
	Range (%)	98-109	95-105	90-96	87-103	100-105	96-104	104-107
1:16	Average % of Expected	103	102	95	96	99	99	105
	Range (%)	96-110	90-112	87-102	93-98	96-104	94-104	102-108

*Samples were diluted prior to assay.

SENSITIVITY

Seventy-seven assays were evaluated and the minimum detectable dose (MDD) of human EMMPRIN ranged from 1.35-9.77 pg/mL. The mean MDD was 2.94 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human EMMPRIN/CD147 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human EMMPRIN in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum* (n=40)	5041	2986-10,044	1270
EDTA plasma* (n=40)	4938	2986-9441	1237
Heparin plasma* (n=40)	4806	3048-10,087	1272
Saliva (n=10)	2925	544-6892	2061
Urine* (n=15)	18,663	5185-50,207	12,906
Human milk* (n=12)	17,700	7495-55,240	12,563

*Samples were diluted prior to assay as directed by the Sample Preparation section.

Cell Culture Supernates:
Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human EMMPRIN.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	101	180
Stimulated (PHA)	329	594

MCF-7 human breast cancer cells were cultured in F12/DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human EMMPRIN, and measured 703 pg/mL.

THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, and 2 mM L-glutamine for one week and stimulated with 100 ng/mL of lipopolysaccharide for 24 hours. An aliquot of the cell culture supernate was removed, assayed for human EMMPRIN, and measured 3418 pg/mL.

G361 human melanoma cells were cultured in McCoy's 5a media supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human EMMPRIN, and measured 59,334 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human EMMPRIN.

The factors listed below were prepared at 50 ng/mL in the appropriate calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human EMMPRIN control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CD44
EGF R
Hyaluronan (high and low molecular weight)
Integrin α3β1
Integrin α5β1
Integrin αLβ2
Integrin αMβ2
Integrin αVβ3
Integrin αVβ6
Integrin αVβ8
MMP-1
MMP-2
MMP-3
MMP-9
uPA
VEGF

Recombinant mouse:

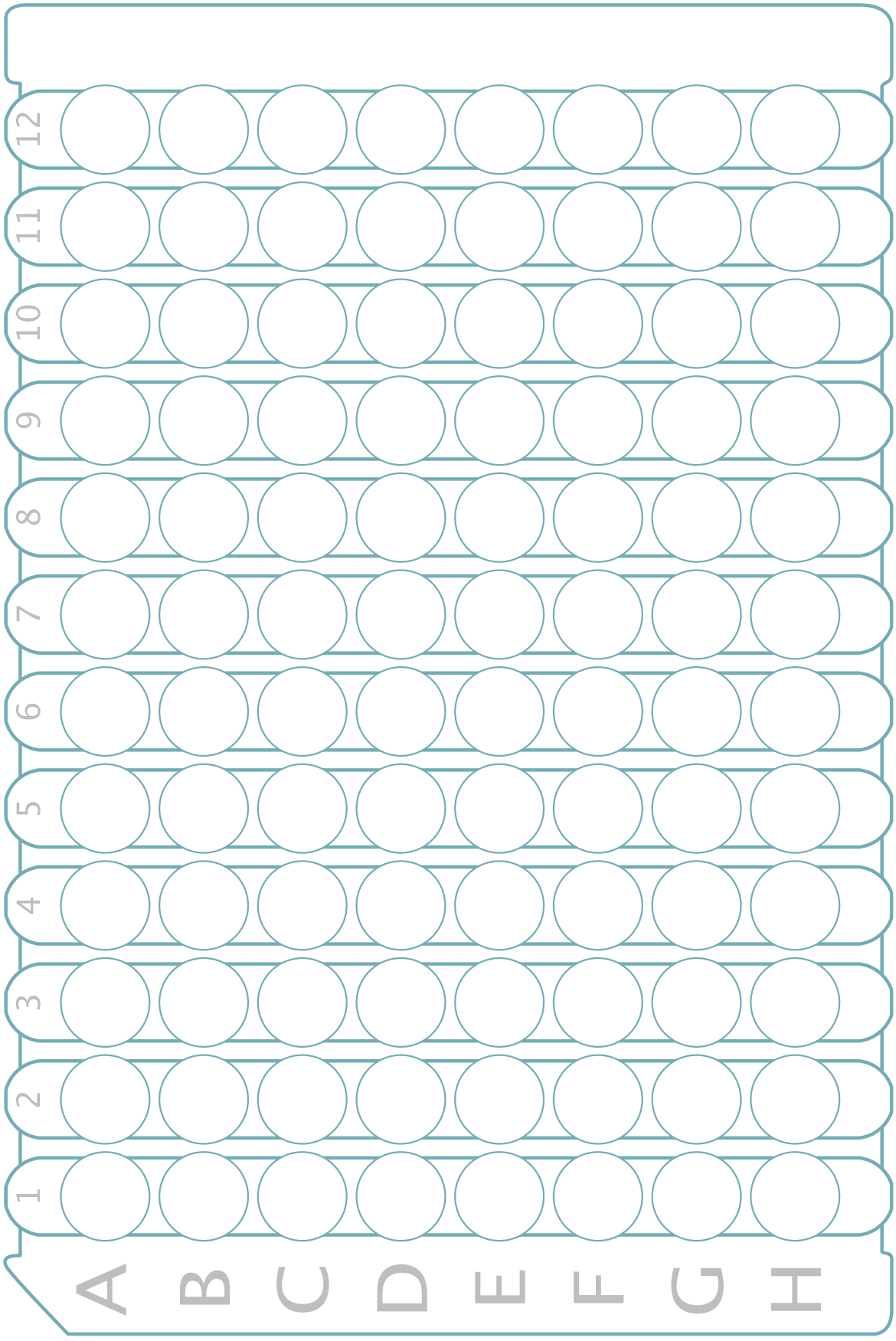
EMMPRIN/CD147

REFERENCES

1. Iacono, K.T. *et al.* (2007) Exp. Mol. Pathol. **83**:283.
2. Hanna, S.M. *et al.* (2003) BMC Biochem. **4**:17.
3. Gabison, E.E. *et al.* (2005) Biochimie **87**:361.
4. Yurchenko, V. *et al.* (2006) Immunology **117**:301.
5. Riethdorf, S. *et al.* (2006) Int. J. Cancer **119**:1800.
6. Braundmeier, A.G. *et al.* (2006) J. Clin. Endocrinol. Metab. **91**:2358.
7. Tang, Y. *et al.* (2006) Mol. Cancer Res. **4**:371.
8. Quemener, C. *et al.* (2007) Cancer Res. **67**:9.
9. Wilson, M.C. *et al.* (2005) J. Biol. Chem. **280**:27213.
10. Xu, D. and M.E. Hemler (2005) Mol. Cell. Proteomics **4**:1061.
11. Tang, W. *et al.* (2004) Mol. Biol. Cell **15**:4043.
12. Kasinrek, W. *et al.* (1992) J. Immunol.**149**:847.
13. Arora, K. *et al.* (2005) J. Immunol. **175**:517.
14. Pushkarsky, T. *et al.* (2005) J. Biol. Chem. **280**:27886.
15. Egawa, N. *et al.* (2006) J. Biol. Chem. **281**:37576.
16. Sidhu, S.S. *et al.* (2004) Oncogene **23**:956.
17. Miyauchi, T. *et al.* (1991) J. Biochem. **110**:770.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES