

Soluble CD147(EMMPRIN) ELISA Kit

INTRODUCTION

CD147, a member of the immunoglobulin superfamily, is a transmembrane glycoprotein included two immunoglobulin-like domains ¹. CD147 is also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or basigin and is enriched on the surface of many malignant tumor cells and stromal cells. The up-regulation of the matrix metalloproteinases (MMPs) by CD147 is believed to be progression of malignancies through tumor growth, invasion and metastasis ^{2,3,4}.

In the recent studies, EMMPRIN fragment (22KDa) is released in a soluble form by the proteolytic cleavage of MMPs from tumor cell surface. There are several evidences that the soluble CD147 is existed into the culture medium of several tumor cells ^{5,6}.

TEST PRINCIPLE

This assay kit employs the quantitative sandwich ELISA technique based on two mouse monoclonal antibodies against to specific different epitope of EMMPRIN. One of specific antibody is pre-coated onto 96-well microplate.

Calibrators and samples (culture medium and clinical sample, e.g. urine) are applied into the wells and incubated, EMMPRIN is captured by the coated antibody. Following, the microplate is incubated with biotinylated antibody. And next step, HRP-conjugated streptavidin is added to the well and incubated. Finally, a chromogenic substrate solution (H₂O₂ and OPD) is applied to each test well and color develops in proportion to EMMPRIN level. The enzymatic reaction is stopped by addition of sulphuric acid and absorbance of the color intensity is measured spectrophotometrically at 490 nm. Calibration curve is constructed, and EMMPRIN concentrations of samples are able to determine using the calibration curve.

KIT COMPONENTS

○ Antibody pre-coated 96-well microplate	--- 1 plate (6 strips)
○ Biotinylated antibody, concentrated (100 x)	--- 1 vial (120 µL)
○ HRP-conjugated streptavidin, concentrated (100 x)	--- 1 vial (120 µL)
○ Antibody diluent	--- 1 vial (30 mL)
○ Wash solution, concentrated (20 x)	--- 1 vial (30 mL)
○ Substrate solution	--- 1 vial (30 mL)
○ OPD tablet	--- 2 tablets
○ Stop solution (sulphuric acid)	--- 1 vial (15 mL)
○ Sample diluent	--- 1 vial (15 mL)
○ EMMPRIN standard (1000 ng/mL)	--- 2 vial (30 µL/vial)

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm.
- Precision (multi-channel) pipettes and disposable tips to deliver 10 – 1000 µL.
- Polypropylene test tubes for diluting samples, calibrators and reagents.
- Graduated cylinder or bottle (500 mL volume) for preparing Wash solution.
- Deionized or distilled water.

PROCEDURAL HINTS

- The kit should be stored at the appropriate condition, and not be used beyond the expiry date.
- Reagents with different lot numbers should not be mixed.
- The thawed reagents should be stored at 2-8°C and used within 1 week.
- The prepared (diluted) reagents should be used within the day.
- Clinical samples should be handled as potentially infectious agents.
- The assay procedure should be continually performed. The wells removed aliquot should not be kept dry.

SAMPLE PREPARATION

Culture medium and clinical sample (e.g. urine) are appropriately diluted by Sample diluent.

REAGENTS PREPARATION

- Biotinylated antibody solution
Dilute 100 µL of concentrated Biotinylated antibody with 10 mL of Antibody diluent.
- HRP-conjugated streptavidin solution
Dilute 100 µL of concentrated HRP-conjugated streptavidin with 10 mL of Antibody diluent.
- Wash solution
Dilute 25 mL of concentrated Wash solution into deionized water to prepare 500 mL of wash solution.
- Chromogenic substrate solution
Dissolve 1 OPD tablet with 13 mL of Substrate solution. Use within a few minutes.
- EMMPRIN calibrators
Dilute 25 µL of EMMPRIN standard with 475 µL of Sample diluent, and constitute 50 ng/mL of EMMPRIN calibrator#1. Following, mix with equal volume (250 µL) of calibrator and Sample diluent, produce a series of 7 calibrators for duplicates (#1; 50 ng/mL, #2; 25 ng/mL, #3; 12.5 ng/mL, #4; 6.25 ng/mL, #5; 3.13 ng/mL, #6; 1.56 ng/mL, #7; 0.78 ng/mL).

ASSAY PROCEDURE

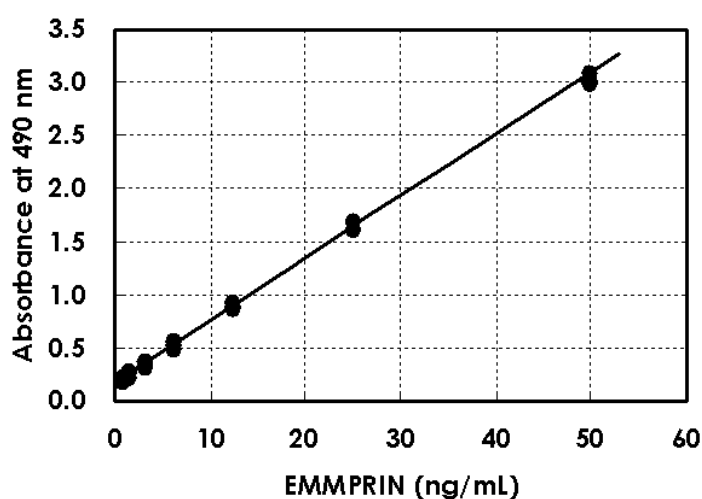
1. Pipet 100 μ L of calibrators and samples into the wells of Antibody pre-coated 96-well microplate and incubate at room temperature (25°C) for 1 hour.
2. Wash the wells 3 times with 300 μ L of Wash solution, and tap the plate for no droplets remaining.
3. Pipet 100 μ L of Biotinylated antibody solution into the wells and incubate at room temperature (25°C) for 1 hour.
4. Perform washing step about 2.
5. Pipet 100 μ L of HRP-conjugated streptavidin solution into the wells and incubate at room temperature (25°C) for 30 min.
6. Perform washing step about 2.
7. Pipet 100 μ L of Chromogenic substrate solution into the wells and incubate at room temperature (25°C) for 10 min.
8. Add 100 μ L of Stop solution into the wells.
9. Measure the optical density of the wells at 490 nm using a microplate reader.

CALCULATION

The EMMPRIN concentrations are recommended to calculate based on the calibration curve using a built-in program of the most microplate readers.

For manual, first find the absorbance value on the Y-axis and extend a horizontal line to the calibration curve. At the point of intersection, extend a vertical line to the X-axis and read the corresponding EMMPRIN concentration.

Typical data; Calibration curve



KIT PERFORMANCE

Sensitivity (the corresponding concentration of blank absorbance + 2SD); 0.30 ng/mL

Intra assay Precision (n = 10); CV < 10%

Inter assay Precision (n = 10); CV < 10%

STORAGE

Store at -30°C.

The kit is stable until the expiry date indicated on the box label.

CAUTIONS

- For research purpose only. Not for in vitro diagnostic use.
- Handle with care the reagents. Especially, avoid contact with sulphuric acid, substrate solution and OPD. Protect your eye, mouth, skin and clothing.
- Follow your local regulation for disposal of all waste materials

REFERENCES

- 1) Yoshida S. *et al.* (2000) *Eur J Biochem* 267, 4372-4380
- 2) Millimaggi D. *et al.* (2007) *Neoplasia* 9(4), 349 – 357
- 3) Kathryn T. *et al.* (2007) *Exp Mol Pathol* 83(3), 283–295
- 4) Reimers N. *et al.* (2004) *Clin Cancer Res* 10, 3442-3428
- 5) Egawa N. *et al.* (2006) *J Biol Chem* 281(49), 37576 – 37585
- 6) Tang, Y. *et al.* (2004) *Mol Cancer Res* 2, 73-80

Supplier

SCETI
SCETI K.K.

3-6-7 Kasumigaseki, Chiyoda-ku, Tokyo 100-0013, JAPAN
Tel +81(3) 5510-2347 Fax +81(3) 5510-0133
URL: <http://www.sceti.jp/export/> e-mail: exp-pet@sceti.co.jp