Code No. 27190

Human N-ERC/Mesothelin Assay Kit - IBL

INTRODUCTION

Erc has been identified as a gene showing stronger expression in cancer-affected renal tissue than in normal renal tissue in Eker rats (a rat model of renal cancer). The human homologue of the protein encoded by this gene is called MPF (megakaryocyte potentiating factor) or mesothelin. This protein is detected especially prominently in mesothelial cells, and its involvement has been suggested in the development of mesothelioma, making it a promising tumor marker. In humans, involvement of this protein has also been suggested in the development of pancreatic, ovarian and pulmonary cancers, etc. The protein is expressed as a GPI anchor-type membranous protein (about 71 kDa in molecular weight), which is digested by a furin-like protease to yield fragments about 31 kDa and 40 kDa in size.
We have established a system for the assay of this protein, referring to the 31 kDa fragment as N-ERC/Mesothelin.
This ELISA kit can measure N-ERC/Mesothelin in human plasma samples.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human N-ERC/Mesothellin.

MEASUREMENT RANGE

0.07 - 4.4 ng/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination Human N-ERC/ Mesothelin in EDTA plasma.

KIT COMPONENT

1	Precoated plate : Anti-Human ERC (7E7) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human ERC (20A2) Mouse IgG MoAb Fab' Affinity Purify	0.4mL x 1
3	Standard (Lyophilized) : Recombinant human N-ERC/Mesothelin	1.0mL x 2
4	EIA buffer	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Microplate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Incubator (37°C ± 1°C)
- Plate washer
- Paper towel
- Tube for dilution of Standard
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

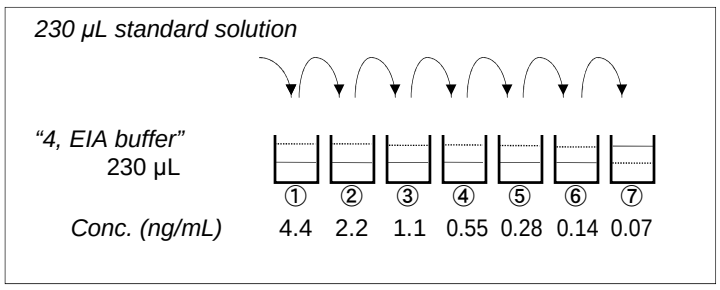
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>1.0 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 8.8 ng/mL Human N-ERC/Mesothelin standard.
4) Dilution of Standard
Prepare 7 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	4.4 ng/mL
Tube-2	2.2 ng/mL
Tube-3	1.1 ng/mL
Tube-4	0.55 ng/mL
Tube-5	0.28 ng/mL
Tube-6	0.14 ng/mL
Tube-7	0.07 ng/mL

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 4.4 ng/mL and 0.07 ng/mL.

See following picture.



5) Dilution of test sample
Dilute specimens more than 8-fold with "4, EIA buffer".

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid			
Aspiration of solution			
Labeled Antibody	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature with plate lid			
5 times (wash buffer more than 350 µL)			
Chromogen	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)			
Stop solution	100 µL	100 µL	100 µL
Read the plate at 450nm/600-650nm against a Test Sample Blank within 30 minutes after addition of Stop solution.			

1) Perform the following procedure on the strips of precoated plate.
2) Determine wells for test sample blank. Put 100 µL of "4, EIA buffer" into the each well.
3) Determine wells for test sample and diluted standard.
Then, put 100 µL each of test sample and diluted standard (tube-1-7) into the appropriate wells.
4) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
5) Aspirate solution from each well of the precoated plate with a plate washer.
6) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
7) Incubate the precoated plate for 30 minutes at room temperature after covering it with plate lid.
8) Wash the plate with the prepared wash buffer and remove all liquid.*
9) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
10) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
11) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
12) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a test sample blank.
In the case of using dual wavelength, dominant wavelength is 450 and reference wavelength is 600-650 nm.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

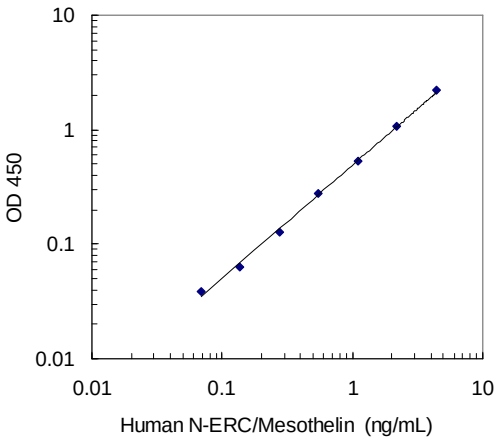
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", more than 8-fold.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer in this kit for washing the precoated plate.
Insufficient washing may lead to the failure in measurement.
6) Washing step after primary incubation is not necessary, but washing about 4 times won't affect the result.
7) In the case of manual washing procedure (with washing bottle), remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
8) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
9) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
(e.g. quadratic regression by double logarithmic plot, refer to the example below).
Using the mean absorbance value for unknown samples determine the corresponding concentration from the standard curve.
For the calculation of the concentrations in initial samples, dilution factor has to be taken into account.

Example of standard curve

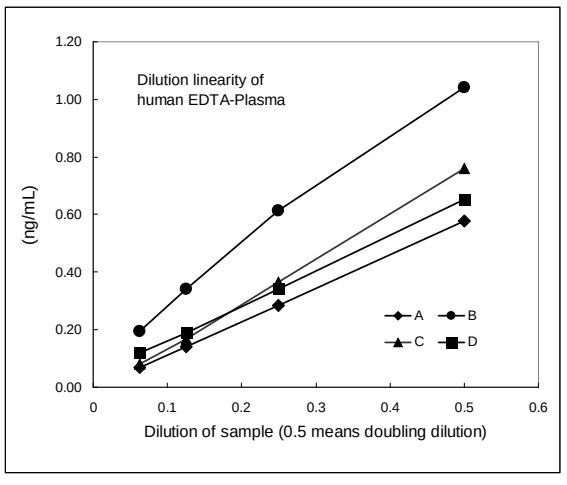
Conc. (ng/mL)	Absorbance (450nm)
4.4	2.191
2.2	1.076
1.1	0.526
0.55	0.279
0.28	0.127
0.14	0.064
0.07	0.039



＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Dilution linearity



2. Intra - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
1.65	0.041	2.5	22
0.48	0.018	3.8	22
0.20	0.011	5.5	22

3. Inter - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
1.66	0.09	5.4	6
0.46	0.03	6.5	6
0.19	0.01	5.3	6

4. Specificity

Substance	Cross-Reactivity
Human N-ERC/Mesothelin	100 %
Human C-ERC/Mesothelin	< 0.1 %
Mouse N-ERC/Mesothelin	< 0.1 %

5. Sensitivity

0.017 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

REFERENCE

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