

Code No. 27765

Rat N-ERC/Mesothelin Assay Kit - IBL**INTRODUCTION**

Erc has been identified as a gene showing stronger expression in cancer-affected renal cell 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 thought to be 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 and 40 kDa fragment as C-ERC/Mesothelin.

The kit, an ELISA kit designed for the assay of N-ERC/Mesothelin, is aimed at analyzing the expression of ERC in detail using tumor cell lines, serum and plasma.

PRINCIPLE

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

MEASUREMENT RANGE

0.08 ~ 5 ng/mL

INTENDED USE

This IBL's assay kit is capable for the quantitative determination rat N-ERC Mesothelin in serum, EDTA plasma, ascites and cell culture supernatant.

KIT COMPONENT

1	Precoated plate : Anti-Rat ERC (30F2) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Rat ERC (280) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Rat N-ERC/Mesothelin	0.5mL 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**

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Incubator (37°C ± 1°C)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

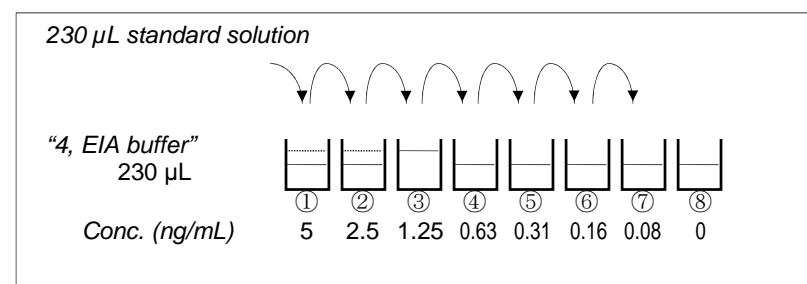
2. Preparation

- 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.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 10 ng/mL Rat N-ERC/Mesothelin standard.
- 4) Dilution of Standard
Prepare 8 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	5 ng/mL
Tube-2	2.5 ng/mL
Tube-3	1.25 ng/mL
Tube-4	0.63 ng/mL
Tube-5	0.31 ng/mL
Tube-6	0.16 ng/mL
Tube-7	0.08 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

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 5 ng/mL and 0.08 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.

**5) Dilution of test sample**

Test sample should be diluted with "4, EIA buffer" as necessary. The recommended dilution for serum and plasma sample is 40 times. In the case of ascites samples, try to start from around 1,000 times dilution.

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	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1~7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
4 times (wash buffer more than 350 µL)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

- 1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
- 3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
- 4) Wash the plate with the prepared wash buffer and remove all liquid.*
- 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid.*
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
- 11) 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 liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

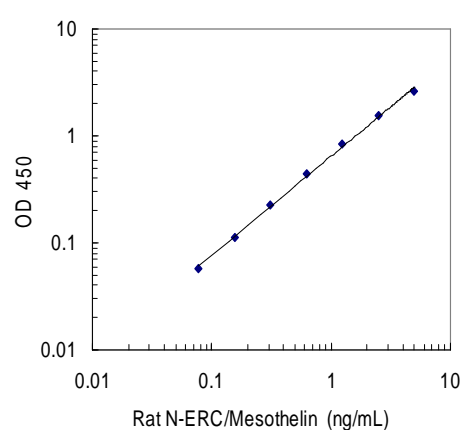
- 1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer", if the need arises.
- 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 contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
- 8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (ng/mL)	Absorbance (450nm)
5	2.586
2.5	1.547
1.3	0.851
0.63	0.450
0.31	0.229
0.16	0.115
0.08	0.060
0 (Test Sample Blank)	0.003



* 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. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
10%FCS added RPMI-1640	8	0.64	0.63	101.6
	16	0.32	0.31	103.2
	32	0.16	0.16	100.0
Rat Serum (SD)	8	2.70	2.84	95.1
	16	1.56	1.57	99.4
	32	1.03	0.95	108.4
Rat Plasma (EDTA) (SD)	8	3.21	3.08	104.2
	16	1.74	1.70	102.3
	32	0.89	0.81	109.9

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10%FCS added RPMI-1640 (x8)	1.25	1.23	98.4
	0.63	0.59	93.7
	0.31	0.27	87.1
Rat Serum (SD) (x8)	3.46	3.01	87.0
	2.84	2.50	88.0
	2.52	2.30	91.3
Rat Plasma (EDTA) (SD) (x8)	3.75	3.64	97.1
	3.12	2.99	95.8
	2.81	2.69	95.7

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
2.15	0.09	4.2	24
1.10	0.05	4.5	24
0.24	0.01	4.2	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
2.19	0.10	4.6	9
1.12	0.05	4.5	9
0.24	0.01	4.2	9

5. Specificity

Compound	Cross Reactivity
Rat N-ERC/Mesothelin	100%
Human N-ERC/Mesothelin	≤0.1%
Rat MCP-1	≤0.1%
Rat TNF-α	≤0.1%
Rat Osteopontin	≤0.1%

6. Sensitivity

0.01 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

- All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
- "3, Standard" is lyophilized products. Be careful to open this vial.
- "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".
- Dispose used materials after rinsing them with large quantity of water.
- Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
- Wash hands after handling reagents.
- Do not mix the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.
- 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

- Hino O, Kobayashi E, Nishizawa M, Kubo Y, Kobayashi T, Hirayama Y, Takai S, Kikuchi Y, Tsuchiya H, Orimoto K, et al. Renal carcinogenesis in the Eker rat. *J Cancer Res Clin Oncol.* 1995;121(9-10):602-5.
- Yamashita Y, Yokoyama M, Kobayashi E, Takai S, Hino O. Mapping and determination of the cDNA sequence of the Erc gene preferentially expressed in renal cell carcinoma in the Tsc2 gene mutant (Eker) rat model. *Biochem Biophys Res Commun.* 2000 Aug 18;275(1):134-40.
- Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet.* 2003 Nov 15;362(9396):1612-6.
- Scholler N, Fu N, Yang Y, Ye Z, Goodman GE, Hellström KE, Hellström I. Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma. *Proc Natl Acad Sci U S A.* 1999 Sep 28;96(20):11531-6.
- Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Soluble mesothelin-related protein--a blood test for mesothelioma. *Lung Cancer.* 2005 Jul;49 Suppl 1:S109-11.
- Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. *Clin Cancer Res.* 2004 Jun 15;10(12 Pt 1):3937-42.
- Onda M, Willingham M, Nagata S, Bera TK, Beers R, Ho M, Hassan R, Kreitman RJ, Pastan I. New monoclonal antibodies to mesothelin useful for immunohistochemistry, fluorescence-activated cell sorting, Western blotting, and ELISA. *Clin Cancer Res.* 2005 Aug 15;11(16):5840-6.
- Hassan R, Remaley AT, Sampson ML, Zhang J, Cox DD, Pingpank J, Alexander R, Willingham M, Pastan I, Onda M. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. *Clin Cancer Res.* 2006 Jan 15;12(2):447-53.
- Shiomi K, Miyamoto H, Segawa T, Hagiwara Y, Ota A, Maeda M, Takahashi K, Masuda K, Sakao Y, Hino O. Novel ELISA system for detection of N-ERC/mesothelin in the sera of mesothelioma patients. *Cancer Sci.* 2006 Sep;97(9):928-32. Epub 2006 Jun 7.
- Maeda M, Hino O. Molecular tumor markers for asbestos-related mesothelioma: serum diagnostic markers. *Pathol Int.* 2006 Nov;56(11):649-54.
- Nakaishi M, Kajino K, Ikesue M, Hagiwara Y, Kuwahara M, Mitani H, Horikoshi-Sakuraba Y, Segawa T, Kon S, Maeda M, Wang T, Abe M, Yokoyama M, Hino O. Establishment of the enzyme-linked immunosorbent assay system to detect the amino terminal secretory form of rat Erc/Mesothelin. *Cancer Sci.* 2007 May;98(5):659-64.
- Kuwahara M, Takeda M, Takeuchi Y, Kuwahara M, Harada T, Maita K. Transforming growth factor beta production by spontaneous malignant mesothelioma cell lines derived from Fisher 344 rats. *Virchows Arch.* 2001 May;438(5):492-7.
- Tsuchiya H, Tsuchiya Y, Kobayashi T, Kikuchi Y, Hino O. Isolation of genes differentially expressed between the Yoshida sarcoma and long-survival Yoshida sarcoma variants: origin of Yoshida sarcoma revisited. *Jpn J Cancer Res.* 1994 Nov;85(11):1099-104.
- Hino O, Shiomi K, Maeda M. Diagnostic biomarker of asbestos-related mesothelioma: Example of translational research. *Cancer Sci.* 2007 Aug;98(8):1147-51.
- Hagiwara Y, Hamada Y, Kuwahara M, Maeda M, Segawa T, Ishikawa K, Hino O. Establishment of a novel specific ELISA system for rat N- and C-ERC/mesothelin. *Rat ERC/mesothelin in the body fluids of mice bearing mesothelioma. Cancer Sci.* 2008 Apr;99(4):666-70.

Version 2.

February 2017 *

Made in Japan.