Code No. 27189

Human HB-EGF Assay Kit - IBL

INTRODUCTION

HB-EGF (Heparin-binding EGF-like growth factor) is a growth factor which belongs to EGF family and it is a ligand of a receptor tyrosine kinase of ErbB family such as EGF receptor and ErbB4. HB-EGF exists as 3 types of form and the forms are transmembrane (proHB-EGF), soluble HB-EGF (sHB-EGF) which has been cleaved by protease at surface of cells and C terminal fragment (HB-EGF-CTF) which is remained at cell membrane after it has been cleaved. These forms of HB-EGF have different physiological activities. HB-EGF expresses in various cells such as epidermal cells, cardiac myocyte, vascular endothelial cells, smooth myocyte and macrophage. HB-EGF which is secreted in blood involves with cells proliferation, differentiation and inflammatory reaction. It has important roles not only maintaining of physiological functions especially in morphogenesis of the development and in pathogenesis. In recent years, it attracts attentions as a drug target of molecularly-targeted drugs because it has been reported that over-expression of HB-EGF is essential for cancer invasion and metastasis.

Human HB-EGF form which contains pro-region can be quantitatively measured by this ELISA kit.

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 HB-EGF.

MEASUREMENT RANGE

0.34 - 22.00nmol/L

INTENDED USE

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

This IBL's assay kit is capable for the quantitative determination Human HB-EGF in serum, EDTA plasma, Urine, CSF and cell culture supernatant.
Recommended dilution ratio for;
(1) Normal human serum and EDTA plasma samples is around 2-fold.
(2) Normal human Urine samples is around 20-fold.
(3) Normal human CSF samples is around 4-fold.

KIT COMPONENT

1	Precoated plate: Anti-Human HB-EGF (45F1) Mouse IgG MoAb		96Well x 1
2	Labeled antibody Conc.:		:
	(30X) HRP conjugated Anti- Human HB-EGF (58B7) Mouse IgG Fab'		0.4mL x 1
3	Standard	: Recombinant Human HB-EGF	0.5mL x 2
4	EIA buffer	: 1% BSA, 0.05% Tween-20 in PBS	30mL x 1
5	Solution for Labeled antibody: 1% BSA, 0.05% Tween-20 in PBS		12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution	: 1N H_2SO_4	12mL x 1
8	Wash buffer Conc.	: (40X) Phosphate buffer	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

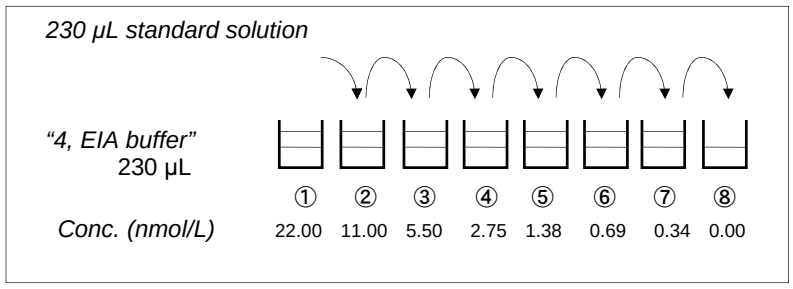
- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

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 1950 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-fold 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 2 - 8°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 44.00 nmol/L Human HB-EGF 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	22.00 nmol/L
Tube-2	11.00 nmol/L
Tube-3	5.50 nmol/L
Tube-4	2.75 nmol/L
Tube-5	1.38 nmol/L
Tube-6	0.69 nmol/L
Tube-7	0.34 nmol/L
Tube-8	0.00 nmol/L (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 22.00 nmol/L and 0.34 nmol/L. Tube-8 is the test sample blank as 0.00 nmol/L.
See following picture.



5) Dilution of test sample
Test samples should be diluted with "4, EIA buffer" suitably.
Recommended dilution ratio for;
(1) Normal human serum and EDTA plasma samples is around 2-fold.
(2) Normal human Urine samples is around 20-fold.
(3) Normal human CSF samples is around 4-fold.

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 O/N at 2 - 8°C with plate lid				
Washing 4 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 60 minutes at 2 - 8°C with plate lid				
Washing 5 times				
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 overnight at 2 - 8°C after covering it with plate lid.
4) Wash each well of the precoated plate 4 times with wash buffer using a washing bottle or a plate washer in following way.
After shaking off (or aspiration of) the solution in wells, fill each well with wash buffer and shake off the wash buffer completely from the precoated plate. <u>This procedure must be repeated 4 times.</u> Then, drain the precoated plate completely on paper towel.
Please refer to 5) and 6) in SPECIAL ATTENION below, and be careful not to miss a well.
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 60 minutes at 2 - 8°C after covering it with plate lid.
7) Wash the precoated plate 5 times in the same manner as 4.
In case of using a plate washer, we recommend manually washing in the manner mentioned above at least the last one time.
8) 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.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) 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".
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 solution. 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", suitably.
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) Remove the wash buffer completely by tapping the precoated plate on paper

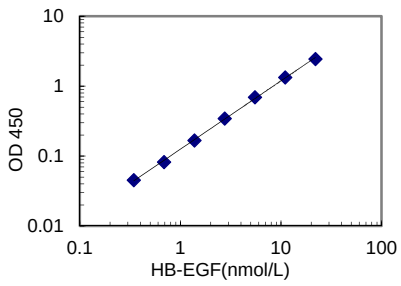
towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

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

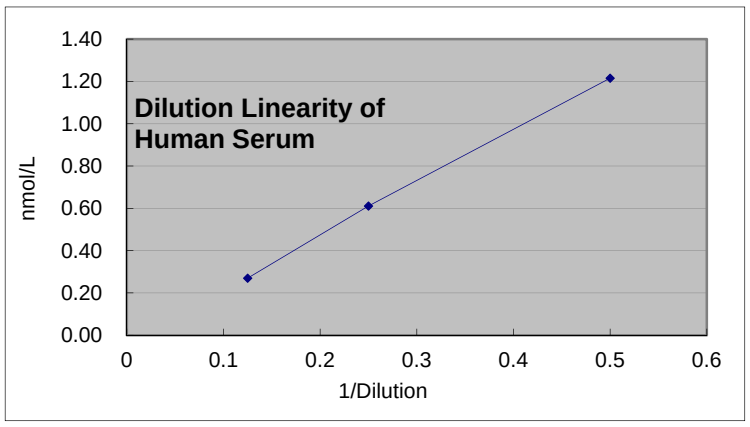
Conc. (nmol/L)	Absorbance (450nm)
22.00	2.462
11.00	1.343
5.50	0.702
2.75	0.350
1.38	0.173
0.69	0.088
0.34	0.051
0.00 (Test Sample Blank)	0.005

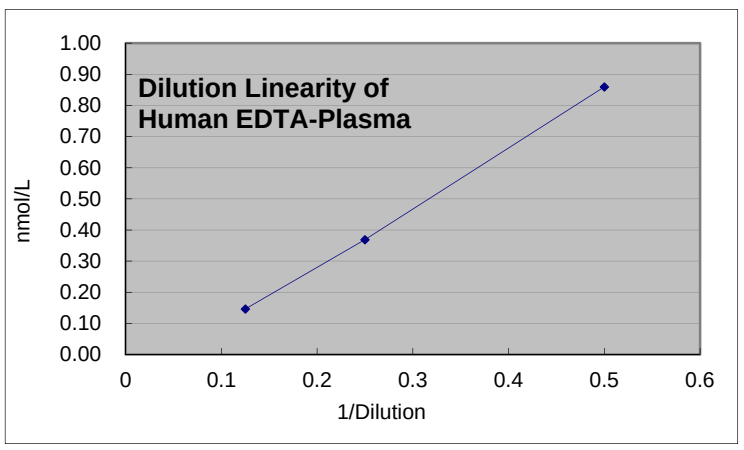


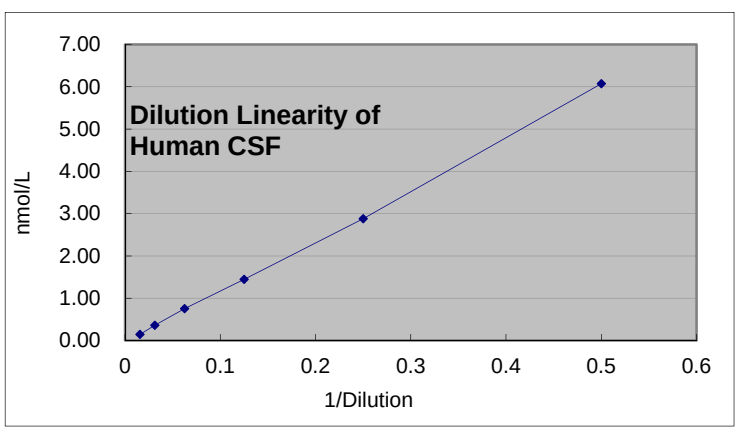
* The typical standard curve is shown above. This curve cannot be used to derive test results. Please run a standard curve for each assay.

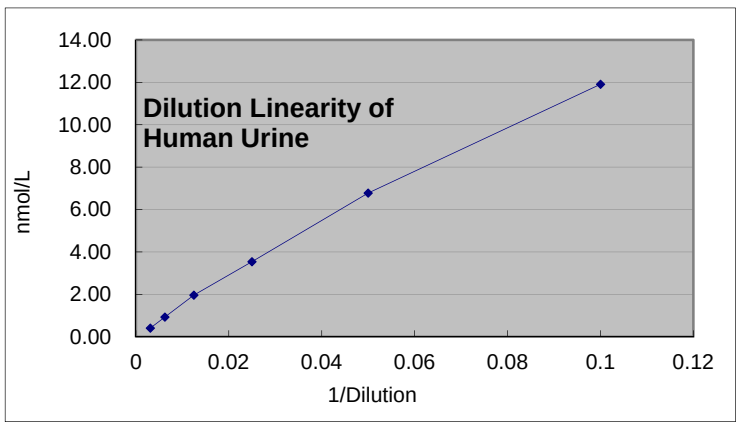
PERFORMANCE CHARACTERISTICS

1. Titer Assay









2. Added Recovery Assay

Specimen	Theoretical Value (nmol/L)	Measured Value (nmol/L)	%
Human Serum X2	12.19	11.86	97.3
	3.94	4.44	112.7
	1.88	2.08	111.0
Human Plasma (EDTA) X2	3.65	3.39	93.0
	1.59	1.72	108.1
	1.07	1.11	103.6
Human Urine X20	10.88	10.85	99.7
	6.76	7.91	117.1
	5.72	6.57	114.8
Human CSF x4	4.71	4.53	96.1
	2.65	3.10	117.0
	2.13	2.26	106.2
Medium (with10%FBS) x2	11.00	10.71	97.4
	2.75	2.93	106.6
	0.69	0.69	100.7

3. Intra - Assay

Mean Value (nmol/L)	SD (nmol/L)	CV (%)	n
6.91	0.19	2.8	24
3.48	0.11	3.3	24
1.77	0.05	2.8	24

4. Inter - Assay

Mean Value (nmol/L)	SD (nmol/L)	CV (%)	n
7.13	0.14	2.0	6
3.72	0.11	2.9	6
1.82	0.06	3.3	6

5. Specificity

Substance	Cross-Reactivity
Human HB-EGF	100%
Human HB-EGF(63-148)	N.D.

6. Sensitivity
0.048nmol/L
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

1. Higashiyama S, Lau K, Besner GE, Abraham JA, Klagsbrun M. Structure of heparin-binding EGF-like growth factor. Multiple forms, primary structure, and glycosylation of the mature protein. J Biol Chem. 1992 Mar 25;267(9):6205-12.

2. Nanba D, Higashiyama S. Dual intracellular signaling by proteolytic cleavage of membrane-anchored heparin-binding EGF-like growth factor. Cytokine Growth Factor Rev. 2004 Feb;15(1):13-9. Review.

3. Ota I, Higashiyama S, Masui T, Yane K, Hosoi H, Matsuura N. Heparin-binding EGF-like growth factor enhances the activity of invasion and metastasis in thyroid cancer cells. Oncol Rep. 2013 Oct;30(4):1593-600.

4. Miyamoto S, Yagi H, Yotsumoto F, Kawarabayashi T, Mekada E. Heparin-binding epidermal growth factor-like growth factor as a novel targeting molecule for cancer therapy. Miyamoto S, Yagi H, Yotsumoto F, Kawarabayashi T, Mekada E. Cancer Sci. 2006 May;97(5):341-7. Review.

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Made in Japan.