Code No. 27769

Human LRG Assay Kit - IBL

INTRODUCTION

Among the diseases which should be distinguished from iNPH (idiopathic normal pressure hydrocephalus) of elderly people showing symptoms such as gait disorder and dementia, there are some neurodegenerative diseases such as Alzheimer's disease (AD), FTLD (frontotemporal lobar degeneration) and disorder associated with Parkinson's disease.
This ELISA kit can measure LRG in CSF, blood or urine. It has become possible to distinguish iNPH from neurodegenerative diseases such as AD by measuring LRG in CSF.

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 Human LRG.

MEASUREMENT RANGE

1.56 ～ 100 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 of Human LRG in serum, EDTA plasma, urine and cerebrospinal fluid.
- The guide line of dilution range for serum and plasma samples is 2,000-fold to 10,000-fold. However, optimal dilution should be examined by each experiment

.

KIT COMPONENT

1	Precoated plate	:	
	Anti-Human LRG (162) Rabbit IgG Affinity Purify		96Well x 1
2	Labeled antibody Conc.	:	
	(30X) HRP conjugated Anti-Human LRG (329) Rabbit IgG Fab' Affinity Purify		0.4mL x 1
3	Standard	: Recombinant Human LRG	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)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Incubator (37°C ± 1°C)
- 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 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.

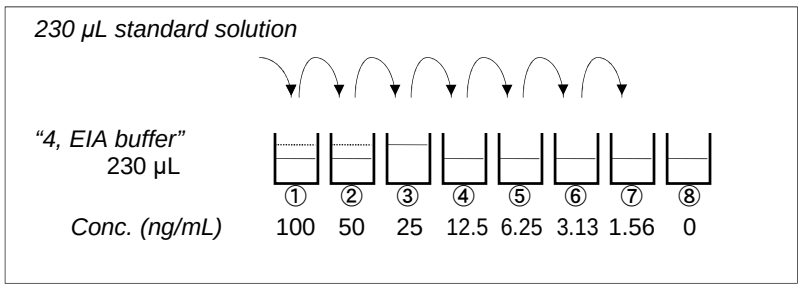
3) Preparation of Standard
Put just 0.5 mL of Deionized water* into the vial of "3, Standard" and mix it gently and completely. This solution is 200 ng/mL Human LRG standard. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

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	100 ng/mL
Tube-2	50 ng/mL
Tube-3	25 ng/mL
Tube-4	12.5 ng/mL
Tube-5	6.25 ng/mL
Tube-6	3.13 ng/mL
Tube-7	1.56 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 100 ng/mL and 1.56 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
Test samples need to be diluted with "4, EIA buffer" supplied with this kit suitably.
When the concentration of human LRG in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.
When "4, EIA buffer" in kit is not enough for dilution, customers can purchase additional EIA buffer for human LRG (100 mL, Code No. 27769D100).

6) Example of sample dilution
10,000-fold dilution of serum or EDTA-plasma
At first, add 990 µL of "4, EIA buffer" to 10 µL of sample and mix it gently and completely. Then, this solution is "100-fold diluted sample".
Next, add 990 µL of "4, EIA buffer" to 10 µL of the "100-fold diluted sample" and mix it again. Then, the resulting solution is 10,000-fold diluted sample and use it for determination.

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 18 hours at 4 °C with plate lid				
Washing 4 times (wash buffer more than 350 µL)				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 37 °C with plate lid				
Washing 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 18 hours at 4 °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 37 °C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid completely.
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 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. Diluted samples should be immediately measured after the dilution.
2) Test samples have to be diluted with "4, EIA buffer" supplied with this kit 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

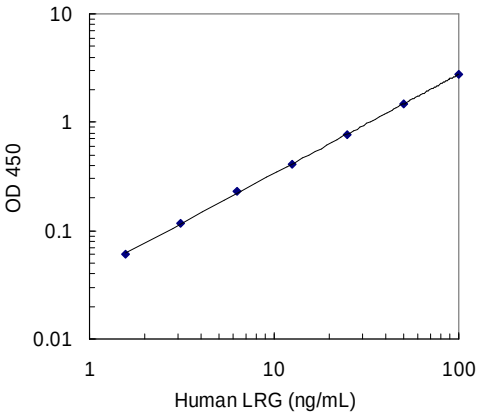
towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

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

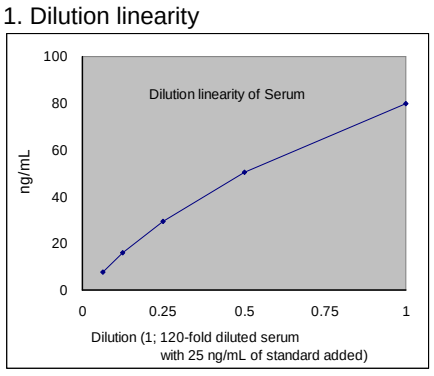
Conc. (ng/mL)	Absorbance (450nm)
100.	2.893
50.	1.577
25.	0.860
12.5	0.507
6.25	0.329
3.13	0.215
1.56	0.160
0 (Test Sample Blank)	0.099

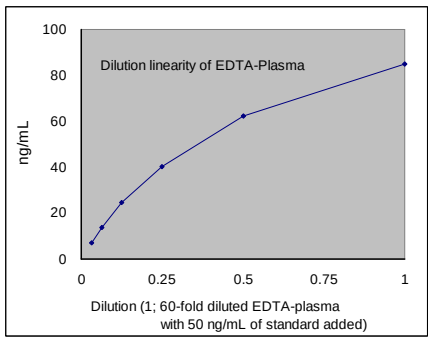


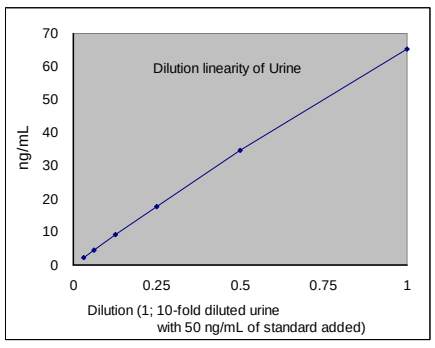
The typical standard curve is shown above. This curve cannot be used to derive test results. Please run a standard curve for each assay.

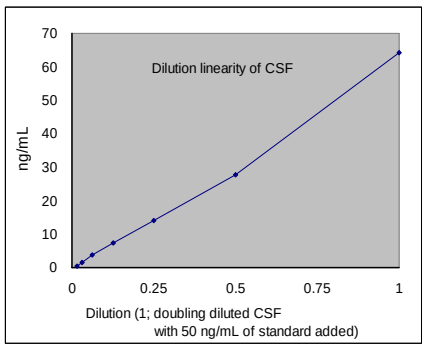
PERFORMANCE CHARACTERISTICS

1. Dilution linearity









2. Added Recovery Assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Human Serum (x100)	12.5	75.78	76.92	101.5
	6.25	69.53	73.72	106.0
	3.12	66.40	70.09	105.5
Human Plasma (EDTA) (x100)	12.5	50.18	50.64	100.9
	6.25	43.93	47.92	109.0
	3.12	40.80	44.90	110.0
Human Urine (X10)	50	66.83	64.86	97.0
	25	41.83	42.07	100.5
	12.5	29.33	29.99	102.2
Human Cerebrospinal fluid (x2)	6.25	24.59	23.84	96.9
	3.12	21.46	21.89	102.0
	1.56	19.90	20.69	103.9

3. Intra – Assay

Measurement Value (ng/mL)	SD (ng/mL)	CV (%)	n
85.34	2.60	3.0	14
22.78	1.05	4.6	14
5.23	0.26	4.9	14

4. Inter – Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
87.30	3.69	4.2	3
22.60	0.59	2.6	3
5.09	0.26	5.1	3

5. Specificity

Compound	Cross Reactivity
Human LRG	100 %
Human Aβ (1-42)	< 0.01%
Human sAPPβ (w)	< 0.01%
Human sAPPα	< 0.01%

6. Sensitivity

0.17 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

1. Li X, Miyajima M, Mineki R, Taka H, Murayama K, Arai H. Analysis of potential diagnostic biomarkers in cerebrospinal fluid of idiopathic normal pressure hydrocephalus by proteomics. Acta Neurochir (Wien). 2006 Aug;148(8):859-64
2. Serada S, Fujimoto M, Ogata A, Terabe F, Hirano T, Iijima H, Shinzaki S, Nishikawa T, Ohkawara T, Iwahori K, Ohguro N, Kishimoto T, Naka T. iTRAQ-based proteomic identification of leucine rich alpha 2 glycoprotein (LRG) as a novel inflammatory biomarker in autoimmune diseases. Ann Rheum Dis. 2010 Apr;69(4):770-4.
3. Nakajima M, Miyajima M, Ogino I, Watanabe M, Miyata H, Karagiozov KL, Arai H, Hagiwara Y, Segawa T, Kobayashi K, Hashimoto Y. Leucine-rich α-2-glycoprotein is a marker for idiopathic normal pressure hydrocephalus. Acta Neurochir. 2011 Jun;153(6):1339-46.
4. Watson CJ, Ledwidge MT, Phelan D, Collier P, Byrne JC, Dunn MJ, McDonald KM, Baugh JA. Proteomic analysis of coronary sinus serum reveals leucine-rich α2-glycoprotein as a novel biomarker of ventricular dysfunction and heart failure. Circ Heart Fail. 2011 Mar 1;4(2):188-97.

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Made in Japan.