

LRG ELISA

Enzyme immunoassay for the quantitative determination of human LRG in CSF, serum, EDTA plasma and urine

REF

JP27769



12 x 8

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used.

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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, FTLD (frontotemporal lobar degeneration) and disorder associated with Parkinson's disease. iNPH can be treated by ventriculo-peritoneal shunt (VP shunt), so a marker in spinal fluid which can distinguish it from neurodegenerative diseases in an early stage has been required. Arai, Miyajima *et al.* had identified the high level of leucine-rich alpha-2-glycoprotein (LRG) in cerebrospinal fluid of iNPH patients, and they reported that measurement of LRG would be useful for differential identification of iNPH.

This ELISA kit can measure LRG in CSF, blood or urine. It has become possible to distinguish iNPH from neurodegenerative diseases such as Alzheimer's disease by measuring LRG in CSF. From now, early diagnosis and therapy of iNPH are expected to lead to the improvement of patient's prognosis.

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

This IBL's assay kit is capable for the quantitative determination Human LRG in serum, EDTA plasma, urine and cerebrospinal fluid.

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 : 1% BSA, 0.05% Tween20 in PBS	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution : 1N H ₂ SO ₄	12mL x 1
8	Wash buffer Conc. : (40X) 0.05% Tween20 in phosphate buffer	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

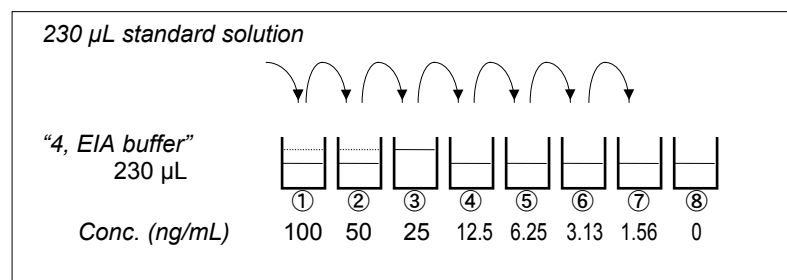
2. Preparation

- 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 "4, EIA buffer" into the vial of "3, Standard" and mix it gently and completely. This solution is 200 ng/mL Human LRG 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	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 sample may be diluted with "4, EIA buffer" as necessary.

If 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.

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 overnight at 4 °C with plate lid				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 37 °C with plate lid				
Washing 9 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 4 °C after covering it with plate lid.
- 4) Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
- 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 precoated plate 9 times in the same manner as 4).
- 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".

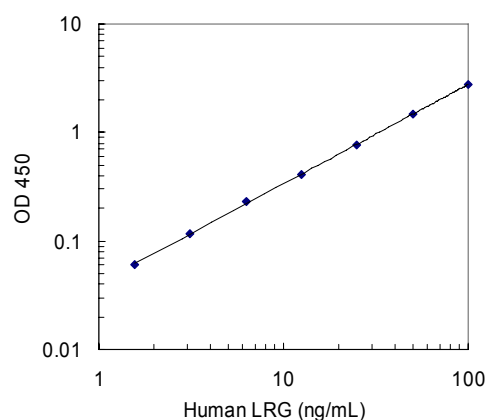
solution".

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 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)	%
Human Serum	120	80.02	86.50	92.5
	240	50.61	53.37	94.8
	480	29.50	30.19	97.7
Human Plasma (EDTA)	60	85.09	102.87	82.7
	120	62.16	64.20	96.8
	240	40.40	36.34	111.1
Human Urine	10	65.40	66.45	98.4
	20	34.60	34.08	101.5
	40	17.71	17.22	102.8
Human Cerebrospinal fluid	2	70.71	64.18	110.1
	4	31.00	27.79	111.5
	8	15.58	14.15	110.1

2. Added Recovery Assay

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

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	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 A β (1-42)	$\leq 0.01\%$
Human sAPP β (W)	$\leq 0.01\%$
Human sAPP α	$\leq 0.01\%$

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

- 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".
- "1, Precoated plate" and "3, Standard" contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
- 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 term of validity : 12 months
 (The expiry date is specified on outer box.)

REFERENCE

- 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

Version. 1

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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