Instructions for Use

GIP total (mouse) High Sensitive ELISA

Enzyme immunoassay for the quantitative determination of mouse GIP total in EDTA-plasma

REF **30116202**

Σ **12 x 8**

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

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Mouse GIP, Total (high sensitivity) Assay Kit - IBL

96 Well

Please read carefully this instruction prior you use this assay kit.

INSTRUCTIONS FOR USE

This product is for research use only and is not intended for diagnostic use.

KIT COMPONENT

1-1	**Streptavidin plate**	96Well x 1
1-2	**Biotinylated Antibody:** Anti-GIP(C) 27B1 Mouse IgG Biotinylated	12mL x 1
2	**Labeled antibody conc.:** (30X) HRP conjugated Anti-GIP (3-17) 81A1 Mouse IgG Fab' A.P	0.4mL x 1
3	**Standard:** GIP (1-42)	0.5mL x 2
4	**EIA buffer**	30mL x 1
5	**Solution for antibody**	30mL x 1
6	**Chromogen:** TMB solution	15mL x 1
7	**Stop solution**	12mL x 1
8	**Wash buffer conc.**	50mL x 1

MEASURING SAMPLES

Mouse EDTA-plasma

PRINCIPLE

This kit is a sandwich ELISA (Enzyme-linked Immunosorbent Assay). As a streptavidin is coated on a plate and biotinylated antibody is added into it to be fixed the capture antibody. Samples and standard are added into the wells for 1st reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

OPERATING PRECATION

1. Test samples should be measured soon after collection. For storage of 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” contained in this kit.
3. Duplicate measurement of test samples and standards is recommended.
4. Standard curve should run for each assay.
5. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6. All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7. Use only “8, Wash buffer conc.” contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8. Fill the wash buffer each well, invert the plate and make sure the liquid is completely removed by shaking it off if you use a washing bottle. Repeat this washing process several times as instructed in order to avoid any insufficient washing process.
9. After remove the wash buffer, tapping the plate against a clean paper towel for completely removing the liquid from the wells and make sure the paper towel is not contact with inside of the wells in this process.
10. “6, Chromogen - TMB solution” should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11. After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
12. Measurement of O.D. should be done within 30 minutes after addition of “7, Stop solution”.

OPERATION MANUAL AND DOSAGES

1. Materials needed but not supplied.

Plate reader	Micropipette and tip
Test tubes for dilution	Measuring cylinder and beaker
Deionized water	Plate washer
Paper towel	Collecting container
Refrigerator	(i.e. clean disposable test tube)

2. Preparation

(1) Preparation of wash buffer

Dilute “8, Wash buffer conc.” 40 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.

(2) Preparation of biotinylated antibody

Add 12mL for “5. Solution of antibody” into “1-2. Biotinylated antibody” and completely dissolve it. This solution is used as dissolved biotinylated antibody for measurement. The dissolved biotinylated antibody can be freeze stored. Freeze and thaw should not be repeated.

(3) Preparation of labeled antibody

Dilute “2, Labeled antibody conc.” 30 fold with “5, Solution for antibody” using a prepared collecting container.

(4) Preparation of standard

Add 0.5 mL of deionized water into the vial of “3, Standard” and completely dissolve it. Concentration of the standard is 200 pmol/L. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.

Prepare 7 test tubes for dilution of the standard and adding 230 µL of the EIA buffer into each tube.

Put 230 µL of 200 pmol/L standard into the tube 100 pmol/L (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 50 pmol/L (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 100 pmol/L and 1.56 pmol/L.

Tube-1	100	pmol/L
Tube-2	50	pmol/L
Tube-3	25	pmol/L
Tube-4	12.50	pmol/L
Tube-5	6.25	pmol/L
Tube-6	3.13	pmol/L
Tube-7	1.56	pmol/L

(4) Preparation of test samples

Dilute test samples with “4, EIA buffer” contained in this kit as follows.

Mouse EDTA-plasma: 20 fold.

3. Measurement Procedure

(1) Add dissolved biotinylated antibody

Prepare necessary slit and add 100µL dissolved biotinylated antibody into each well.

(2) Coat the dissolved biotinylated antibody with plate lid

(3) Washing

Wash the plate with the prepared wash buffer and remove all liquid.

(4) Add test sample blank

Determine wells for test sample blank. Put 100µL each of “4, EIA buffer” into the wells.

(5) Add prepared test samples and standard

Put 100 µL prepared test samples and 100 µL prepared standard into appropriate wells.

(6) Incubation with plate lid (1st reaction).

(7) Washing

Wash the plate with the prepared wash buffer and remove all liquid.

(8) Add prepared labeled antibody

Put 100 µL prepared labeled antibody into the wells.

(9) Incubation with plate lid (2nd reaction).

(10) Washing

Wash the plate with the prepared wash buffer and remove all liquid completely.

(11) Add “6, Chromogen - TMB solution”

Put 100 µL the TMB solution into the wells.

(12) Incubation in dark

(13) Add “7, Stop solution”

Put 100 µL the Stop solution into the wells.

(14) Determination of optical density (O.D.)

Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, measure the both O.D. of standard and the test samples against a test sample blank.

Measurement wavelength: 450 nm. In case of 2 wavelengths:

Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

Table for measurement procedure

Dissolved biotinylated antibody	100µL		
Coating	Incubation for 60 minutes at R.T. (shielded).		
Washing	2 times (wash buffer more than 350 µL)		
	Test samples	Standard	Test sample blank
Reagents	Test samples 100 µL	Test samples 100 µL	Test samples 100 µL
1st reaction	Incubation for Overnight at 2 ~8°C with plate lid.		
Washing	4 times (wash buffer more than 350 µL)		
Labeled antibody	100 µL	100 µL	100 µL
2nd reaction	Incubation for 60 minutes at 2 ~8°C with plate lid.		
Washing	5 times (wash buffer more than 350 µL)		

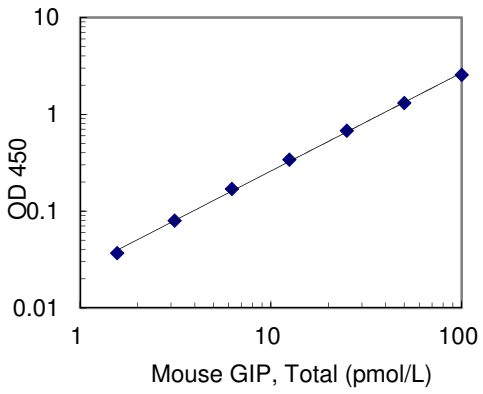
TMB solution	100 µL	100 µL	100 µL
Chromogenic reaction	Incubation for 30 minutes at R.T. (shielded).		
Stop solution	100 µL	100 µL	100 µL
Measuring O.D.	450 nm / 600～650 nm		

CALCULATION OF TEST RESULT

1 Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. quadratic regression of double logarithm conversion).
2 Read the concentration by applying the absorbance of the test samples on a standard curve.
3 Calculate the concentration of the test samples by multiplying dilution ratio of test samples on the value.

Example of standard curve and measured value

Standard (pmol/L)	O.D. (450nm)
100	2.556
50	1.308
25	0.677
12.50	0.340
6.25	0.170
3.13	0.080
1.56	0.037

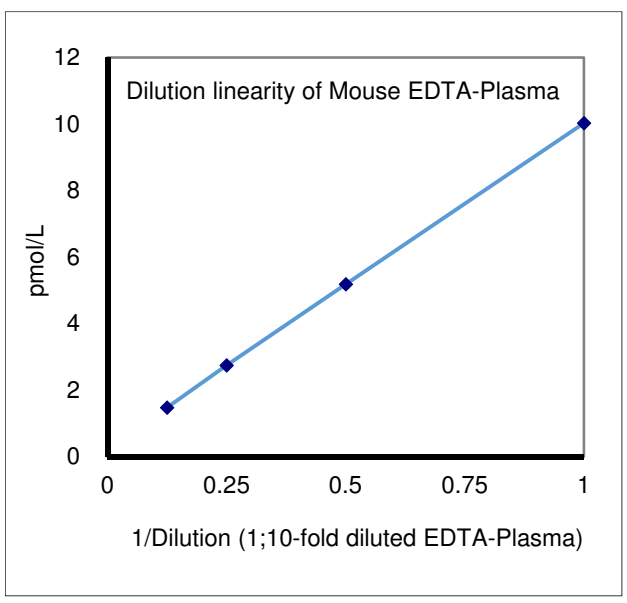


PERFORMANCE AND CHARACTERISTICS

1 Sensitivity
0.84 pmol/L

2 Measurement range
1.56 ～ 100 pmol/L

3 Dilution linearity



4 Added recovery assay

Specimen	Additive Amount (pmol/L)	Theoretical Value (pmol/L)	Measurement Value (pmol/L)	%
Mouse EDTA-Plasma x20	50.00	53.80	50.27	93.4
	12.50	16.30	15.24	93.5
	3.13	6.93	6.61	95.4

5 Intra-assay

Measurement value (pmol/L)	SD (pmol/L)	CV (%)	n
48.67	1.53	3.1	24
13.29	0.45	3.4	24
4.06	0.28	6.8	24

6 Inter-assay

Measurement value (pmol/L)	SD (pmol/L)	CV (%)	n
44.59	1.98	4.4	7
11.44	0.41	3.6	7
3.59	0.10	2.9	7

7 Specificity

Substance	Cross reactivity (%)
Mouse GIP(1-42)	100
Mouse GIP(3-42)	100
GLP-1(7-36)amide	≦0.1
GLP-1(9-36)amide	≦0.1
Glucagon	≦0.1
Oxyntomodulin	≦0.1

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1 Precaution for handling (Hazard prevention)

(1) Treat the components carefully and wash hands after handling it.
(2) “7, Stop solution” is a strong acid substance (1N Sulfuric acid). Therefore, it should be careful for the treatment and do not contact your skin and clothes with it. It also needs to pay attention to the disposal of it.

2 Precaution for intended use

(1) “3, Standard” is lyophilized products. It should be careful to open this vial.
(2) All reagents should be stored at 2 - 8°C.
(3) Precipitation can be seen in “4, EIA buffer”, “5, Solution for antibody” and “8, Wash buffer conc.”, however, it does not affect its performance.
(4) Do not mix or replace the reagents with the reagents from a different lot or kit.
(5) Do not use expired reagents.

3 Precaution for disposal

(1) Dispose used materials after rinsing them with large quantity of water.

STORAGE AND THE TERM OF VALIDITY

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

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 27701

CONTACT DETAILS

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TEL : 0274-22-2889
FAX : 0274-23-6055

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	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:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	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 evaluazione. / 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: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

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.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

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