GLP1R (RAT) ELISA Kit

Catalog Number KA7464

1 Kit

Version: 01

Intended for research use only

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Table of Contents

Introduction 3

Intended Use 3

Principle of the Assay 3

General Information 4

Materials Supplied 4

Storage Instruction 4

Materials Required but Not Supplied 5

Precautions for Use 5

Assay Protocol 6

Reagent Preparation 6

Sample Preparation 7

Assay Procedure 8

Data Analysis 9

Calculation of Results 9

Performance Characteristics 9

Resources 10

Troubleshooting 10

Plate Layout 12

Introduction

Intended Use

This Sandwich kit is for the accurate quantitative detection of Rat Glucagon-Like Peptide 1 Receptor (also known as GLP1R) in serum, plasma, cell culture supernatants, Ascites, tissue homogenates or other biological fluids.

Principle of the Assay

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat GLP1R antibody. GLP1R present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Rat GLP1R Antibody is added and binds to GLP1R in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated GLP1R antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat GLP1R. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

General Information

Materials Supplied

List of component

Component	Amount
Pre-coated ELISA plate	96 (8x12) wells
Standard solution (3200 ng/L)	0.5 ml
Standard diluent	3 ml
Streptavidin-HRP	6 ml
Stop solution	6 ml
Substrate solution A	6 ml
Substrate solution B	6 ml
Wash buffer Concentrate (25x)	20 ml
Biotinylated Rat GLP1R antibody	1 ml
Plate sealer	2 pics

Storage Instruction

Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

Materials Required but Not Supplied

- ✓ 37°C ± 0.5°C incubator
- ✓ Absorbent paper
- ✓ Precision pipettes and disposable pipette tips
- ✓ Clean tubes
- ✓ Deionized or distilled water
- ✓ Microplate reader with 450 ± 10 nm wavelength filter

Precautions for Use

- ✓ Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- ✓ This instruction must be strictly followed in the experiment.
- ✓ Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- ✓ Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- ✓ Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- ✓ Avoid using the reagents from different batches together.
- ✓ Substrate solution B is sensitive to light, don’t expose substrate solution B to light for a long time.
- ✓ Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- ✓ The kit should not be used beyond the expiration date.

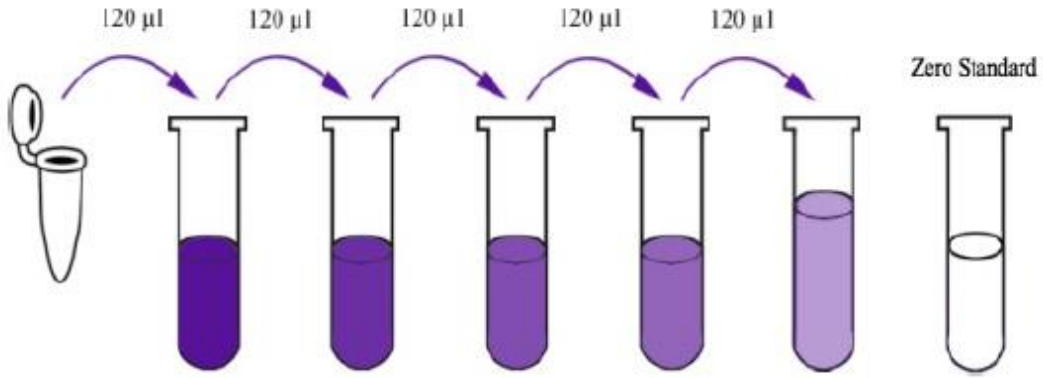
Assay Protocol

Reagent Preparation

✓ All reagents should be brought to room temperature before use.

✓ Standard Reconstitute the 120 µL of the standard (3200 ng/L) with 120 µL of standard diluent to generate a 1600 ng/L standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1600 ng/L) 1:2 with standard diluent to produce 800 ng/L, 400 ng/L, 200 ng/L and 100 ng/L solutions. Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

1600 ng/ml	Standard No.5	120 µL Original standard + 120 µL Standard diluent
800 ng/ml	Standard No.4	120 µL Standard No.5 + 120 µL Standard diluent
400 ng/ml	Standard No.3	120 µL Standard No.4 + 120 µL Standard diluent
200 ng/ml	Standard No.2	120 µL Standard No.3 + 120 µL Standard diluent
100 ng/ml	Standard No.1	120 µL Standard No.2 + 120 µL Standard diluent



Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
3200 ng/ml	1600 ng/ml	800 ng/ml	400 ng/ml	200 ng/ml	100 ng/ml

✓ Wash Buffer Dilute 20 ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Sample Preparation

- ✓ Serum: Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.
- ✓ Plasma: Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.
- ✓ Cell culture supernatant: Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.
- ✓ Tissue: Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant. Avoid freeze/thaw cycles.
- ✓ Urine/Ascites/Cerebrospinal fluid Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Note

1. *Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for user's particular experiment.*
2. *Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 3 months. Avoid repeated freeze thaw cycles.*
3. *Samples should be brought to room temperature before starting the assay.*
4. *Centrifuge to collect sample before use.*
5. *Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).*
6. *Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.*
7. *Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.*

**Sample can't be diluted with this kit. Owing to the material we use to prepare the kit, the sample matrix interference may falsely depress the specificity and accuracy of the assay.*

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50 µL standard to standard well.
   Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40 µL sample to sample wells and then add 10 µL Mouse GLP1R antibody to sample wells, then add 50 µL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300 µL wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50 µL substrate solution A to each well and then add 50 µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 µL Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

✓ Summary

1. Prepare all reagents, samples and standards.
2. Add sample and ELISA reagent into each well. Incubate for 1 hour at 37°C.
3. Wash the plate 5 times.
4. Add substrate solution A and B. Incubate for 10 minutes at 37°C.
5. Add stop solution and color develops.
6. Read the OD value within 10 minutes.

Data Analysis

Calculation of Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Performance Characteristics

✓ Precision

- Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intraassay precision. We measured random samples of KA7463 within the same batch/lot to ensure the consistency of the kits' performances.

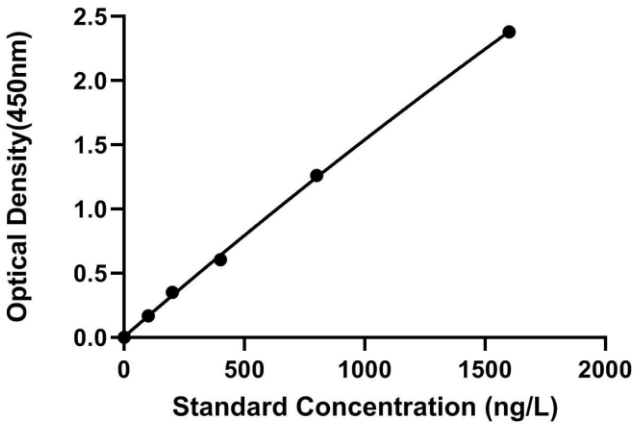
Intra-assay	Sample	n	Mean	Standard deviation	CV (%)
Intra-Assay	1	18	911.8	28.46	3.1
Intra-Assay	2	18	462	22.28	4.8
Intra-Assay	3	18	267	13.37	5

- Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
  CV(%) = SD/mean x 100
  Inter-Assay: CV< 10%

✓ Typical Data

The standard curve of KA7464 is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

KA7464 GLP1R (Rat) Standard Curve



Concentration	O.D	Average	Corrected
1600 ng / ml	2.422	2.414	2.38
	2.406		
800 ng / ml	1.198	1.295	1.261
	1.393		
400 ng / ml	0.659	0.64	0.606
	0.622		
200 ng / ml	0.331	0.384	0.35
	0.437		
100 ng / ml	0.203	0.203	0.169
	0.202		
0 ng / ml	0.036	0.034	0
	0.033		

Resources

Troubleshooting

	Possible case	Solution
High Background possible case	Improper washing	Increasing duration of soaking steps
	Incorrect incubation temperature	Incubate at 37°C
	Incubation time too long	Reduce incubation time
	Substrate exposed to light prior to use	Keep substrate in a dark place
	Substrate was contaminated	Replace substrate. Substrate should be clean and avoid crossed contamination by using the sealer
	Contaminated wash buffer	Use a clean buffers and sterile filter
Weak or No Signal possible case	A reagent or a step of the procedure omitted by mistake	Check protocol and follow steps carefully
	Antibody are not enough	Increase the concentration of the antibody
	Improper washing	Increasing duration of soaking steps
	Reagent are contaminated	Use new one
	Pipette are not clean	Pipette should be clean
	HRP was not added	Add HRP according to the instruction

	Sample contains sodium azide	Don't prepare samples with sodium azide
	Wrong incubation time or temperature	Check and follow protocol. Place plates in an incubator during incubation periods (set to 37ºC).
Poor Precision possible case	Pipetting error	Dispense quickly and identically into the side of each well. Use calibrated pipettes.
	Incomplete washing	Make sure wells are washed adequately by filling the wells with proper amount of wash buffer.
	Unclean wells	Inspect wells and remove debris prior to use. Wipe the outer bottom of plate clean to remove any debris or fingerprints prior to reading.
Poor standard curve possible case	Incorrect preparation of standard	Reconstitute standard as suggested on data sheet.
	Capture Antibody did not bind	Use Abnova ELISA plate in the kit
	Inefficient washing	Be sure wash apparatus is working properly (i.e. distributing even volumes into each well). Be sure wells are empty after aspiration, yet be sure to fill wells in a timely manner.
	Pipetting error	Dispense quickly and identically into the side of each well. Use calibrated pipettes.
	Incorrect storage of components	Store all components as recommended on data sheet. Do not allow reconstituted reagents to stay at room temperature for excess time.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H