EAGLE BIOSCIENCES

Mouse / Rat Active GLP-1 ELISA Assay Kit

Catalog Number:
GP121-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

The primary amino acid sequence of GLP-1 peptide is identical among mammalian species, i.e. rat, mouse, pig, human, etc. The Eagle Biosciences Mouse / Rat Active GLP-1 ELISA Assay Kit (enzyme-linked immunoassay kit) is intended quantitative determination of bioactive glucagon-like peptide-1 (7-36) amide [GLP-1 (7-36)] level in rat and mouse plasma samples with only 20 µl of sample volume. Eagle Biosciences Mouse / Rat Active GLP-1 ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Mouse / Rat Active GLP-1 ELISA Assay Kit is designed, developed and produced for the quantitative measurement of bioactive GLP-1 (7-36) in rodent plasma where there is usually have limited amount of sample available for analysis. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 (7-36) specific antibodies.

Assay standards, controls and test samples are directly added to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 (7-36) specific antibody and a horseradish peroxidase (HRP) conjugated GLP-1 (7-36) specific antibody is added to each well. After the first incubation period, a "sandwich" immunocomplex of "Streptavidin – Biotin-Antibody – GLP-1(7-36) – HRP conjugated antibody" is formed and attached to the wall of the plate. The unbound HRP conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to GLP-1 (7-36) on the wall of the microtiter well is directly proportional to the amount of GLP-1 (7-36) in the sample.

REAGENTS: Preparation and Storage

This Mouse / Rat Active GLP-1 ELISA Assay Kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until the expiration date.

Allow all reagents of the Mouse / Rat Active GLP-1 ELISA Assay Kit to come to room temperature prior to use. Reagents from different kit lot numbers should not be combined or interchanged.

1. Streptavidin Coated Microplate

One well-breakable microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Rodent Active GLP-1 ELISA Assay Kit box.

2. GLP-1 Tracer Antibody

One vial containing 0.6 mL HRP labeled Anti-GLP-1 specific antibody in a stabilized protein matrix. This reagent must be mixed with GLP-1 (7-36) Capture Antibody and the tracer antibody diluent before use (for details see Assay Procedure). This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

3. **GLP-1 (7-36) Capture Antibody**
One vial containing 0.6 mL of biotinylated GLP-1 (7-36) specific antibody. It should be used only after mixed with GLP-1 Tracer Antibody and the tracer antibody diluent according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

4. **ELISA Wash Concentrate**
One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide and non-mercury based preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the Rodent Bioactive GLP-1 ELISA Assay Kit box.

5. **ELISA HRP Substrate**
One bottle contains 12 mL of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

6. **ELISA Stop Solution**
One bottle contains 12 mL of sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

7. **GLP-1 Standards**
Seven vials containing different levels of lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. **Refer to vial for exact concentration for each standard.** These reagents should be stored at 2 – 8 °C and are stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

8. **GLP-1 Controls**
Two vials containing different levels of lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. Refer to vials for exact concentration range for each control. Both controls should be stored at 2 – 8°C and are stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

9. **Tracer Antibody Diluent**
One vial containing 12 mL ready to use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the Mouse / Rat Active GLP-1 ELISA Assay Kit box.

SAFETY PRECAUTIONS

The Mouse / Rat Active GLP-1 Assay Kit reagents must be used in a professional laboratory environment and is for Research Use Only and is not to be used in diagnostic procedures. Only source material from which reagents of bovine serum was derived in the contiguous 48 United States. It was obtained only from donor health animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle

these reagents as if they were potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices**.**

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 20 µL, 50 µL, 100 µL, and 1000 µL etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass/plastic tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA plate shaker
- ELISA multi-channel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
- DPP-4 Inhibitor

SPECIMEN COLLECTION

1. No special preparation of animal is necessary prior to specimen collection. However, fasting sample and non-fasting/glucose induced sample may present great significance for bioactive GLP-1 (7-36) level.
2. **BD™ P700** Blood Collection and Preservation System (contains a DPP-4 protease inhibitor cocktail) must be used for sample collection.
3. As an alternative to BD™ P-700 tubes, whole blood should be collected into a lavender top Vacutainer® EDTA-plasma tube. It is very important to immediately add appropriate amount of DPP-4 inhibitor to the collected EDTA whole blood immediately after the collection (**within 30 seconds**). Refer to DPP-4 inhibitor manufacturer's instruction. Invert tube several times to mix well and place the tube in an ice bath. Centrifuge the tube at 1000 g for 10 minutes in a refrigerated centrifuge..
4. Plasma samples should be stored at 2 – 8 °C if they will be tested within 3 hours of collection. For longer storage, it is recommended to store the plasma sample at -70°C. Aliquot samples before freezing if necessary.

Reagent Preparation

(1) Prior to use allow all reagents of the Mouse / Rat Active GLP-1 ELISA Assay Kit to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) Reconstitute all standards and controls by adding **1.0 mL** of demineralized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix

well by gentle vortexing. These reconstituted standards and controls must be stored at -20°C or below. Do not exceed 3 freeze-thaw cycles.

Test Sample Preparation
For direct measuring Active GLP-1 (7-36), BD™ P-700 Blood Collection and Preservation System must be used for sample collection. There is no other sample preparation necessary prior to assay.

Assay Procedure

1. Place a sufficient number of streptavidin coated microwell strips/wells in a holder to run GLP-1 (7-36) standards, controls and unknown samples in duplicate.
2. Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	C II
B	STD 1	STD 5	C II
C	STD 2	STD 6	SAMPLE 1
D	STD 2	STD 6	SAMPLE 1
E	STD 3	STD 7	SAMPLE 2
F	STD 3	STD 7	SAMPLE 2
G	STD 4	C I	SAMPLE 3
H	STD 4	C I	SAMPLE 3

3. Prepare GLP-1 (7-36) Antibody Mixture: mixing GLP-1 Tracer Antibody and Capture Antibody by 1:21 fold dilution of the Tracer Antibody (30487) and by 1:21 fold dilution of the biotinylated Capture Antibody (30488) with the Tracer antibody Diluent. For each strip, it is required to mix 1 mL of the Tracer Antibody Diluent (30489) with 50 µL the Capture Antibody and 50 µL of the Tracer Antibody in a clean test tube.
4. Add **20 µL** of standards, controls and test samples into the designated microwell.
5. Add **100 µL** of GLP-1 (7-36) Antibody Mixture to each well
6. Cover the plate with one plate sealer and incubate plate at 2-8 °C, static for **20 - 24 hours**.
7. Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of ELISA HRP Substrate into each of the wells.
9. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
10. Incubate plate at room temperature, static for **20 min**.
11. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
12. Read the absorbance at wavelength **450 nm/620 nm or 450 nm/650 nm** within 10 minutes in a microplate reader

PROCEDURAL NOTES
1. Failure to collect samples as above may return erroneous results due to endogenous DPP-4 activity.
2. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate sample should be used for data reduction and the calculation of results.

3. For samples with higher than level 7 standard, it is recommended to measure diluted the specimen with an appropriate GLP-1 free buffer matrix (e.g. standard zero) for a more accurate measurement.
4. Keep light sensitive reagents in the original amber bottles.
5. Store any unused streptavidin coated strips in the foil zipper bag with desiccant to protect from moisture.
6. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
7. Incubation times or temperatures other than those stated in this insert may affect the results.
8. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
9. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.
10. For linearity test, optimal dilution buffer matrix should be used to achieve satisfactory linear recovery.
11. Different dilution buffer matrix used for calibrator or rodent sample may show different linear recovery.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbances of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results. We recommend using **Point-to-Point or log-log curve fit.**

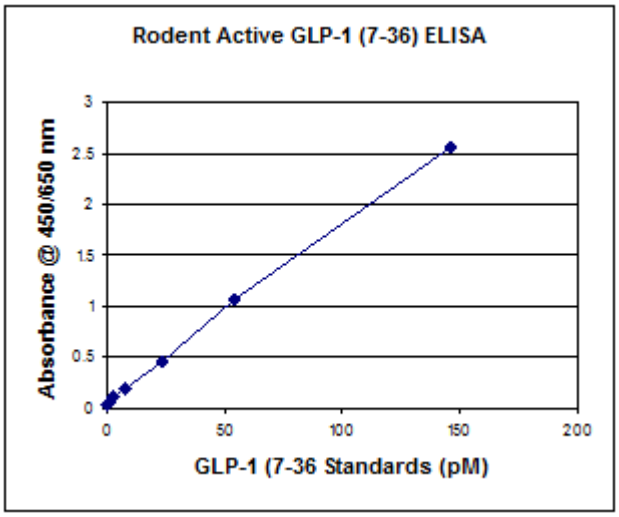
The GLP-1 (7-36) concentrations for the controls and test samples are read directly from the standard curve using their respective corrected absorbance. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 2nd standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd STD)}} \times \text{Value of the 2nd STD}$$

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this GLP-1 ELISA are represented. The example curve was generated using a point-to-point curve fit with linear axes. Other curve fits using linear or logarithmic axes may also be used. **This example curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm/650 nm Absorbance			Results (pM)
	Readings	Average	Corrected	
0 pmol/mL	0.029 0.030	0.030	0.000	
0.85 pM	0.065 0.064	0.065	0.036	
2.4 pM	0.111 0.104	0.108	0.078	
7.9 pM	0.200 0.192	0.196	0.166	
24 pM	0.414 0.516	0.465	0.435	
54 pM	1.051 1.075	1.063	1.033	
146 pM	2.539 2.583	2.561	2.531	
Control I	0.161 0.161	0.161	0.131	5.74
Control II	0.310 0.365	0.337	0.307	16.35



EXPECTED VALUES

Each laboratory should establish it's own normal range by using samples collected from normal healthy animals. Please note that the normal range may be varied by using fasting samples vs. non-fasting samples.

GLP-1(7-36) pg/ml = GLP-1 (7-36) pM x 3.298

Based on limited number of normal donor rodent samples (n = 9), we found the fasting normal range is about 0.5 – 3.1pM and the fed normal range is about 0.6 – 16.7 pM. The table below

shows that in general the Active GLP-1 (7-36) is higher in fed than fasting samples in normal donors.

Donor#	Active GLP-1 (7-36) pM Fasting	Donor#	Active GLP-1 (7-36) pM Fed
1	0.65	10	2.67
2	1.84	11	3.24
3	2.10	12	2.16
4	0.19	13	0.76
5	1.21	14	0.69
6	0.27	15	0.11
7	0.39	16	1.02
8	0.64	17	1.09
9	0.05	18	0.49

LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for GLP-1 (7-36) measurement, the values of assay standards were established using a highly purified GLP-1 (7-36) peptide. Results obtained with different assay methods or kits cannot be used interchangeably.
2. For unknown sample value read directly from the assay that is greater than assay standard level-7, it is recommend measuring a diluted sample for more accurate measurement.
3. Bacterial or fungal contamination of plasma specimens or reagents, or cross contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known GLP-1 (7-36) levels.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the rodent Active GLP-1 (7-36) ELISA is determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.1 pM.

Specificity

This Bioactive GLP-1 (7-36) assay is specific measure GLP-1 (7-36). It is expected that this assay does not detect following peptides.

GLP-1 (7-36) 100%
GLP-1 (9-36) < 0.1%
GLP-1 (9-37) < 0.1%
GLP-1 (7-37) < 0.1%
GLP-1 (1-36) < 0.1%
GLP-2 < 0.1%
Glucagon < 0.1%

Precision

The intra-assay precision was determined by 8 replicates for two control samples in a single assay. A very satisfactory within assay CV% was obtained as indicated below.

#	Average GLP-1 (7-36) pM (n = 12)	SD	CV
Sample 1	4.09	0.274	6.7%
Sample 2	20.56	1.625	7.9%

The inter-assay precision was determined by 6 separate assays on different days with two control samples. The result for between assay CV% was observed as indicated below.

#	Average GLP-1 (7-36) pM (n = 4)	SD	CV
Sample 1	5.53	0.50	9.1%
Sample 2	16.73	0.94	5.6%

Spike Recovery

Rat plasma samples were spiked with 5-20 pM GLP-1 peptide, and the spike recovery was calculated. Please note that matrix used to prepare the spiked samples may exhibit matrix effects.

Sample (pM)	Spike (pM)	Measured value (pM)	Expect value (pM)	Recovery (%)
2.676	5.0	7.42	7.68	96.7
	10.0	11.27	12.68	88.9
	20.0	19.97	22.68	88.0
3.944	5.0	7.38	8.94	82.5
	10.0	13.94	13.89	99.6
	20.0	24.59	23.94	102.7

Linearity
Two rat plasma samples were diluted with GLP-1 (7-36) standard matrix at various percentages. These diluted samples were measured in this assay and the linear recovery was calculated.

Sample 1

Sample / matrix	GLP-1 pM	% Recovery
100% / 0%	107.89	100.0%
90% / 10%	88.20	90.8%
80% / 20%	82.26	95.3%
70% / 30%	65.26	86.4%
60%/ 40%	55.99	86.5%
50% / 50%	44.91	83.3%
40% / 60%	39.16	90.7%
30% / 70%	30.54	94.3%
20% / 80%	22.00	101.9%
10% / 90%	10.33	95.8%

Sample 2

Sample / matrix	GLP-1 pM	% Recovery
100% / 0%	65.50	100%
90% / 10%	54.67	92.7%
80% / 20%	53.69	102.5%
70% / 30%	49.24	107.4%
60%/ 40%	41.52	105.7%
50% / 50%	34.78	106.2%
40% / 60%	30.21	115.3%
30% / 70%	24.45	124.5%
20% / 80%	15.39	117.5%
10% / 90%	6.90	105.3%

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.