GLP-1 Solid Phase Sample Extraction Kit

Catalog Number:
GP100-50
For Research Use Only. Not for use in diagnostic procedures.
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INTENDED USE

This Eagle Biosciences GLP-1 Solid Phase Sample Extraction Kit is for extraction of GLP-1 (Glucagon like peptide 1) from serum or plasma sample. Following the extraction, sample GLP-1 concentration may be measured by using GLP-1 immunoassays. The Eagle Biosciences GLP-1 Solid Phase Sample Extraction Kit is for research use only and not used for diagnostic procedures.

BACKGROUND

Immunoassays were developed for measuring serum GLP-1 level. However, many studies have found that the direct measurement of GLP-1 concentration in serum generates unreliable test results, which are not meaningful clinically and do not correlate at all among multiple immunoassays. Previous studies have suspected that there is an unknown matrix effect for measuring GLP-1 in serum sample by using immunoassays. Dai, et al. reported that solid phase extraction of serum sample before GLP-1 immunoassay eliminates the matrix effect and gives excellent GLP-1 test results, which include (1) clinical meaningful fast/fed GLP-1 serum concentration from normal donors and (2) excellent correlation among three different immunoassays for GLP-1 in serum. Therefore, it is essential to perform a sample extraction before assaying GLP-1.

There are several extraction methods available. Most methods require expensive and special laboratory equipment, such as a vacuum centrifuge. This kit provides a well-validated solid phase GLP-1 sample extraction method by using a very reliable Peptide Extraction cartridge with excellent recovery and reproducibility. The procedure is easy to perform, and well-suited for managing a large number of samples without special equipment.

REAGENTS: Preparation and Storage

The Eagle Biosciences GLP-1 Solid Phase Sample Extraction Kit contains enough reagents and cartridges for extracting 50 specimens. The kit is 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 this expiration date.

1. Peptide Extraction Cartridge

Five pouches, each has 10 cartridges in 3 cc or 60 mg configuration. It contains a unique sorbent, which is a copolymer designed to have a Hydrophilic-Lipophilic Balance. The cartridges should be stored at room temperature or 2-8°C in the sealed pouch and are stable until the expiration date on the kit box. The open pouched cartridge should be used within 4 weeks. The cartridge is for single use only

2. GLP-1 Extraction Buffer A

One bottle containing 65 mL of GLP-1 sample extraction buffer A. It is ready to use. This reagent should be stored at room temperature or 2-8°C and is stable until the expiration date on the kit box.

3. GLP-1 Extraction Buffer B

One bottle containing 125 mL of GLP-1 sample extraction buffer B. It is ready to use. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box.

4. GLP-1 Washing Buffer C

One bottle containing 200 mL of GLP-1 sample washing buffer C. It is ready to use. This reagent should be stored at room temperature or 2-8°C and is stable until the expiration date on the kit box.

5. GLP-1 Elution Buffer D

One bottle contains 50 mL of GLP-1 sample elution buffer D. It is ready to use. This reagent should be stored at room temperature or 2-8°C and is stable until the expiration date on the kit box.

6. GLP-1 Sample Reconstitution Buffer E

One bottle contains 30 mL of GLP-1 sample reconstitution buffer E. It is ready to use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 250 µL, 375 µL, 500 µL, 750 µL, and 1000 µL etc.
2. Polypropylene Peg Rack from Nalgene: Format: 5x10 for 14 mm O.D. tubes (NNI No. 5977-0017; VWR Cat # 60985-415).
3. Elution Collection Tube: polypropylene test tube at 17 x 100 mm (O.D. x L).
4. Evaporating Tube: screw cap polypropylene centrifuge tube at 2 mL volume.
5. Dry Block Heater: It should fit the 2 ml centrifuge tube.
6. Pressure regulated nitrogen tank and manifold: it should fit to the Dry Block Heater.
7. Fume hood.

The above materials and equipment are suggested for use in the GLP-1 sample extraction procedure. Similar materials and equipment may be substituted based on availability and each laboratory's operation.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for research use only. 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.

SPECIMEN COLLECTION & STORAGE

Although both serum and EDTA-plasma samples can be used for measuring Active GLP-1 (7-36) using this assay, It is recommended to use EDTA-plasma, because the plasma sample showed a better stability than serum sample.

(1) Only 375 µL of plasma or serum is required for extraction if Active GLP-1 (7-36) is measured in duplicate. However, a 750 µL sample is required for extraction if both Active GLP-1 (7-36) and Total GLP-1 are measured in duplicate.
(2) No special preparation of individual 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.
(3) Whole blood should be collected into a lavender top Vacutainer® EDTA-plasma tube. Invert tube to mix well and place the tube on ice. Centrifuge the tube within an hour at 1000 g for 10 minutes in a refrigerated centrifuge.
(4) EDTA-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. Avoid more than three freeze-thaw cycles. Aliquot samples before freezing if necessary.

(5) Add appropriate amount of DPP-IV inhibitor to the collected specimen right after the separation of plasma from the blood cells. It is very critical to add DPP IV inhibitor to the collected sample, which stop the degradation of GLP-1 (7-36) to (9-36). Refer to DPP-IV manufacturer's instruction.

(6) BD™ P700 or P800 Blood Collection and Preservation System (contains a DPP-IV protease inhibitor cocktail) is recommended. However, each institute should verify the collection system for their specific research and study.

ASSAY PROCEDURE

1. Reagent Preparation
   (1) All reagents and buffers provided in this kit are ready to use.
   (2) Prior to use allow all reagents and the specimens to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

2. **Extraction Procedure**
   (1) Mark cartridges Elution Collection Tubes and Evaporating Tubes with numbers correlated to samples to be extracted. Place cartridges on Nalgene Peg Rack and place the rack in a pan. The extraction buffer and waste are allowed to flow freely into the pan.
   (2) Add 1 mL of GLP-1 Extraction Buffer A to each cartridge and allow the buffer A to drip through by gravity.
   (3) When all of Buffer A has entered the sorbent, add 1 mL of GLP-1 Extraction Buffer B to each cartridge and allow the Buffer B to drip through by gravity.
   (4) Repeat step (3) once.
   (5) When all Buffer B has entered the sorbent, load 375 µL serum or plasma sample on correlated cartridge.
   NOTE: 750 µL sample should be loaded on to each cartridge, if two immunoassays will be performed for each specimen after sample extraction.
   (6) Allow sample to drip through by gravity. It will take about 5– 15 minutes.
   (7) Once the entire sample has entered the sorbent, add 1 ml of GLP-1 Washing Buffer C to each cartridge and allow the Buffer C to drip through by gravity.
   (8) Repeat step (7) two more times.
   (9) When all Buffer C has entered the sorbent, place each cartridge onto a corresponding Elution Collection Tube, then add 0.75 mL of GLP-1 Elution Buffer D to each cartridge and allow the Buffer D to drip through by gravity. Make sure that the entire elution buffer is collected with the last drop at the tip of each cartridge.
   (10) Pipet transfer the eluent into correspondent Evaporating Tube.
   (11) Place all above Evaporating Tubes to the Dry Block heater with temperature set at 37°C. The eluent is evaporated under a nitrogen stream at 37 °C for 3 – 5 hours. The evaporated samples may be capped and stored at –20°C for 24 hours.
   (12) Reconstitute each tube with 250 µL of GLP-1 Sample Reconstitution Buffer E and mix thoroughly before running GLP-1 assay.
   NOTE: Reconstitute each tube with 500 µL Buffer E, if 750 µL sample was loaded on to each cartridge.

PROCEDURAL NOTES

1. Usually, each elution step takes less than 5 minutes. During the dripping through procedure, cartridges may be centrifuged at 250 – 500 g to push the reagents or sample through if samples have not dripped though by gravity in 15 min.
2. Alternative extraction procedures may be used to replace the gravity dripping by using a Manifold with SPE vacuum pump (Waters Catalog # WAT200609, 72500417 or 725400418) and other accessories. Please contact Eagle Biosciences for more information.
3. Alternatively, the extracts may be dried overnight under a nitrogen stream at 37°C.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Avoid air bubbles in the extraction cartridge as this could result in slower dripping.
6. All reagents should be mixed gently and thoroughly prior use. Avoid foaming.

QUALITY CONTROL

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

Warranty Information

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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 617-419-2019.