Version 28-02/25

User's Manual

PTH (1-84) Depletion Kit

REF DEIABL239D

Σ 10 x 96 tests

RUO

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

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

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PRODUCT INFORMATION

Intended Use

The PTH (1-84) Depletion Kit is designed for the depletion of PTH (1-84) from serum and plasma.

Principles of Testing

This assay employs an ELISA capture technique. An antibody against PTH (1-84) is coated onto a 96-well microplate. Quality Control (QC) and Test Samples are pipetted into the appropriate wells. PTH (1-84) in the QC and Test Samples binds to the antibody coated on the plate. The supernatant is collected for further analysis.

Reagents And Materials Provided

□ Coating Protein (2000X): Store at -15 to -30ºC upon receipt. Avoid repeated freeze-thaw.

□ Blocking Buffer

□ Positive Control Concentrate (1 mg/mL): Store at -60 to -80ºC upon receipt. Avoid repeated freeze-thaw.

Materials Required But Not Supplied

•Microplate Reader (450-620 nm)

•Plate Shaker

•Distilled or De-Ionized Water

•Disposable Pipette Tips

•Precision Pipettes (5-1000 µL)

•Vortex Mixer

•Multi-Channel Pipette (50-200 µL)

•Adhesive Plate Sealers

•Coating Buffer (Sodium Carbonate/Sodium Bicarbonate Buffer)

•Control Diluent (Human Serum)

•Wash Buffer (Phosphate Buffered Saline with Tween® 20)

Storage

Store kit components at 2 to 8°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials may contain a small amount of reagent. Spin tubes on pulse setting prior to opening. Do not mix or substitute reagents with those from other lots.

Specimen Collection And Preparation

This kit is compatible with EDTA, heparin, citrate plasma, and serum.

Serum: Collect serum with a serum separator tube and allow the whole blood to clot for 25-35 minutes. Centrifuge blood for 15 minutes at 1000 x g and remove serum immediately.

Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Within 30 minutes of whole blood collection, centrifuge for 15 minutes at 1000 x g and remove plasma immediately.

Sample Storage: Store samples immediately after collection, or aliquot and store at or below -20°C for up to one year. Avoid repeated freeze-thaw. It is strongly recommended each lab generate their own stability data.

Avoid using samples with gross hemolysis or lipemia.

Reagent Preparation

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. Coating Solution (1X): Dilute Coating Protein with Coating Buffer 1/2200 before use (for example, add 6 µL Protein (2200X) to 11.994. mL of Buffer for a final volume of 12 mL at 1X). Mix well. Prepare fresh on the day of use.

2. Positive Control: It is advised to make a Sub-Stock of Positive Control at 0.1 mg/mL. Do this by diluting the Positive Control Concentrate with 0.1% Bovine Serum Albumin in Distilled or De-Ionized Water. The Sub-Stock should be aliquoted in single use vials and stored at -60 to -80°C. Avoid repeated freeze-thaws.

a.Intermediate I: Dilute Sub-Stock (0.1 mg/mL) to 1000 ng/mL with Control Diluent (for example, 5 µL of Sub-Stock (0.1 mg/mL) to 495 of Diluent for 1000 ng/mL in 500 µL). Mix well. Do not store excess solution.

b.Intermediate II: Dilute prepared Intermediate I (1000 ng/mL) to 10,000 pg/mL with Control Diluent (for example, 5 µL of Intermediate I (1000 ng/mL) to 495 of Diluent for 10,000 pg/mL in 500 µL). Mix well. Do not store excess solution.

c.Positive Control: Dilute prepared Intermediate II (10,000 pg/mL) to 100 pg/mL with Control Diluent (for example, 14 µL of Intermediate II (10,000 pg/mL) to 1386 of Diluent for 100 pg/mL in 1400 µL). Mix well. Do not store excess solution.

d.Negative Control: This assay uses Control Diluent as Negative Control

Assay Procedure

Step 1. Add 100 µL of prepared Coating Solution (1X) to the plate. Incubate for approx. 2 hours at RT, shaking at approx. 300 rpm.

Step 2. Discard plate contents and tap dry on paper towel. Add 200 µL Blocking Buffer to the plate. Incubate for approx. 1 hour at RT, shaking at approx. 300 rpm.

Step 3. Discard plate contents and wash 3x with 300 µL of Wash Buffer per well. Tap dry on paper towel.

Step 4. Add 100 µL of Controls/Samples to the plate. Incubate for approx. 1 hour at 36 to 38˚C, shaking at approx. 300 rpm. Alternatively, Controls/Samples can be incubated overnight (16 – 20 hours) at 2 to 8°C, shaking at approx. 300 rpm.

Step 5. Collect the supernatant and utilize for desired analysis.

Precautions

1. The test protocol must be followed strictly.
2. All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
3. Some reagents within the kit may contain antimicrobial agents. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
4. Any liquid brought into contact with potentially infectious material needs to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
5. Only trained laboratory personnel should execute this test.