Human Betacellulin, BTC ELISA Kit

Prod. No.: DEIA089
Pkg.Size: 96T

INTENDED USE

This assay is a sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). It is developed for quantitative measurement of human BTC in serum, plasma and other biological fluids.

PRINCIPLE OF THE TEST

An antibody specific for human BTC is coated onto the wells of the microtiter plate. Samples and standards of human BTC are pipetted into the wells for binding to the coated antibody. After washing procedure to remove unbound compounds, an enzyme-linked antibody specific for human BTC is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human BTC bound in the initial step. The color development is stopped and the intensity of the color is measured. The magnitude of the absorbance for this developed color is proportional to the amount of human BTC
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SPECIFICITY&SENSITIVITY

Specificity: This assay recognizes human BTC . No significant cross-reactivity or interference was observed.
Detection Rage: 0.312 ng/ml-10 ng/ml
Detection Limit: The minimum detectable dose of human BTC is typically less than 0.05 ng/ml.

REAGENTS AND MATERIALS PROVIDED

Human BTC Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human BTC ;
Standard (freeze dried): 10 ng/ml, 2 vials;
Biotin-antibody (100×): 120ul;
HRP-avidin (100×): 120ul;
Sample Diluent: 15ml, 2 vials;
Antibody Diluent: 12ml;
HRP-avidin Diluent: 12ml;
TMB Solution A: 10ml;
TMB Solution B: 1ml;
TMB Stop Solution: 12ml;
Wash Buffer (25×): 20ml.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

STORAGE

Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.
Opened/Reconstituted Reagents: Unmixed TMB Solution A; Unmixed TMB Solution B; TMB Stop Solution; Wash Buffer; Sample Diluent; Antibody Diluent; HRP-avidin Diluent. The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

PRECAUTIONS

1. The kit should be equilibrated to room temperature (20-23° C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 25X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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RECONSTITUTION AND STORAGE

Sample: Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.
Standard: Centrifuge the standard vial at 6,000-10,000rpm for 30s. Reconstitute the standard with 1.0 ml of Sample Diluent. The undiluted standard serves as the high standard (10 ng/ml). The Sample Diluent serves as the zero standard (0 ng/ml). Prepare fresh for each assay. Prepare within 2 hours of use.
Biotin-antibody: Centrifuge the vial before opening. Dilute to the working concentration using Biotin-antibody Diluent (1:100), respectively. Prepare within 1 hour of use.
HRP-avidin: Centrifuge the vial before opening. Dilute to the working concentration using HRP-avidin Diluent(1:100), respectively. Prepare within 1 hour of use.
TMB Substrate: TMB Solution A and TMB Solution B should be mixed together at a ratio of 9:1. Prepare within 0.5 hour of use.
Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 25× Wash Buffer Concentrate into deionized or distilled water.

ELISA PROTOCOL

1. Prepare all reagents, working standards, and samples as directed in the previous sections. Dilute original density Standard as follow: Set up 7 points of diluted standard such as 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.312 ng/ml and 0.156 ng/ml. The last EP tubes with Sample Diluent is the blank as 0 ng/ml.

2. Add 100µl of Standard, Control or Sample per well. Cover with the adhesive strip. Incubate for 1.5 hours at 37°C.

3. Aspirate each well and wash, Wash by filling each well with 1 x Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Repeating the process twice for a total of three washes. After the last wash, remove any remaining 1× Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100µl of Biotin-antibody working solution to each well. Incubate for 1 hour at 37°C. Biotin-antibody working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

5. Repeat the Aspirate/Wash three times.

6. Add 100µl of HRP-avidin working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 0.5 hours at 37°C.

7. Repeat the Aspirate/Wash five times.

8. Add 100ul TMB Substrate to each well. Mix gently, **protected from light** and incubates at 37°C for 15-20 min.

9. Add one drop (100 µl) of TMB Stop Solution to each well to stop the color reaction. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the sample concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding sample concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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