Version 07-11/23

User's Manual

Human Bone Marrow Stromal Cell Antigen 1, BST1 ELISA Kit

REF **CKERS-BST1-229H**

Σ **5 plates**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The human BST1 ELISA kit is for the quantitative determination of human BST1.

This ELISA kit contains the basic components required for the development of sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on five 96-well plates.

Principles of Testing

The ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for BST1 coated on a 96-well plate. Standards and samples are added to the wells, and any BST1 present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-BST1 monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of BST1 present in the sample streptavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450nm.

Storage

Keep streptavidin-HRP at 4°Cand protect it from pro-longed exposure to light. Aliquot all other reagents and store at -20°C to -70°C in a manual defrost freezer.

Reconstitution And Storage

Bring all reagents to room temperature before use.

Capture Antibody: 0.5 mg/ml of mouse anti-BST1 monoclonal antibody. Dilute to a working concentration of 1 µg/ml in CBS before coating.

Detection Antibody: 0.3 mg/ml of biotinylated rabbit anti-BST1 polyclonal antibody. Dilute to a working concentration of 0.2 µg/ml in detection antibody dilution buffer before use.

Standard: Each vial contains 15 ng of recombinant BST1. Reconstitute with 1 ml detection antibody dilution buffer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 1 ng/ml is recommended.

Streptavidin-HRP: 50µl of streptavidin conjugated to horse-radish-peroxidase. 1: 2,000 Dilution in detection antibody dilution buffer before use.

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100uL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300µl of blocking buffer to each well. Incubate at room temperature for a minimum of

1hour.

4. Repeat the aspiration/wash as in step2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100µl of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100µl of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100µl of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200µl of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
8. Add 50µl of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

Calculation

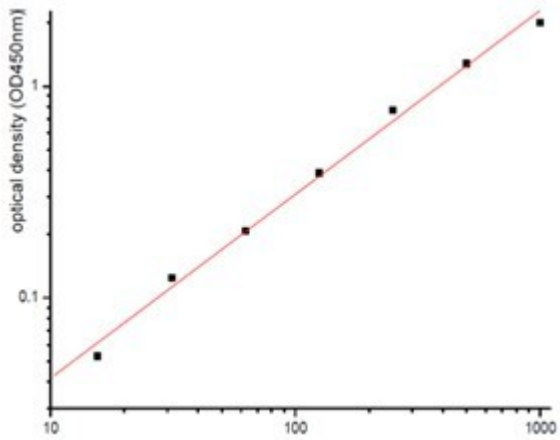
Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

BST1 concentration(pg/ml)



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
15.6	0.036
31.25	0.064
62.5	0.134
125	0.233
250	0.465
500	0.823
1000	1.333

Sensitivity

15.6 pg/mL

Precautions

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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