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User's Manual

Human CCL19 ELISA kit

REF **DEIA3273**

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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 CCL19 (Human) ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human MIP-3 beta in biological samples, such as serum, plasma, cell culture supernatants, urine, and/or cell and tissue lysates.

Principles of Testing

This assay employs an antibody specific for human MIP-3 beta coated on a 96-well plate. Standards and samples are pipetted into the wells and MIP-3 beta present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human MIP-3 beta antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MIP-3 beta bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. Antibody-coated ELISA Plate (Item A)-96 wells (12 strips x 8 wells) coated with specific capture antibody.
2. 20x Wash Buffer (Item B)-RABWASH4: 25 mL of 20x concentrated solution.
3. Target Protein Standard (Item C)-2 vials, recombinant protein.
4. Assay/Sample Diluent Buffer/s: See current Certificate of Analysis
5. Biotinylated Detection Antibody (Item F)-2 vials of biotinylated detection antibody (each vial is enough to assay half a microplate).
6. HRP-Streptavidin (Item G)-RABHRP5: 200 µL of concentrated HRP-conjugated streptavidin.
7. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H)-RABTMB3: 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
8. ELISA Stop Solution (Item I)-RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Software which can perform four-parameter logistic regression models.
8. Tubes to prepare standard or sample dilutions.

Storage

Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25°C) before use.
2. Assay/Sample Diluent Buffer dilution: See current Certificate of Analysis for dilution instructions.
3. Sample dilution: See current Certificate of Analysis for dilution instructions and recommendations.

Note: Levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of target protein standards: See current Certificate of Analysis for dilution instructions.
5. If the Wash Buffer (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.
6. Preparation of Biotinylated Detection Antibody: See current Certificate of Analysis for dilution instructions.
7. Dilution of HRP-Streptavidin concentrate (Item G): See current Certificate of Analysis for dilution instructions.

Assay Procedure

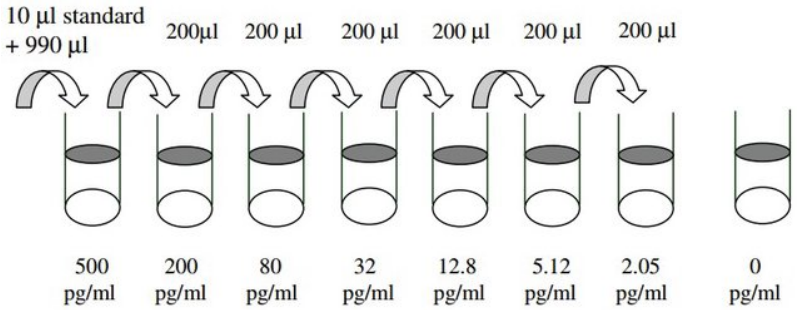
1. Bring all reagents and samples to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of each standard (see Reagent Preparation, step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of 1x prepared Biotinylated Detection Antibody (see Reagent Preparation, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µL of ELISA Colorimetric TMB Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

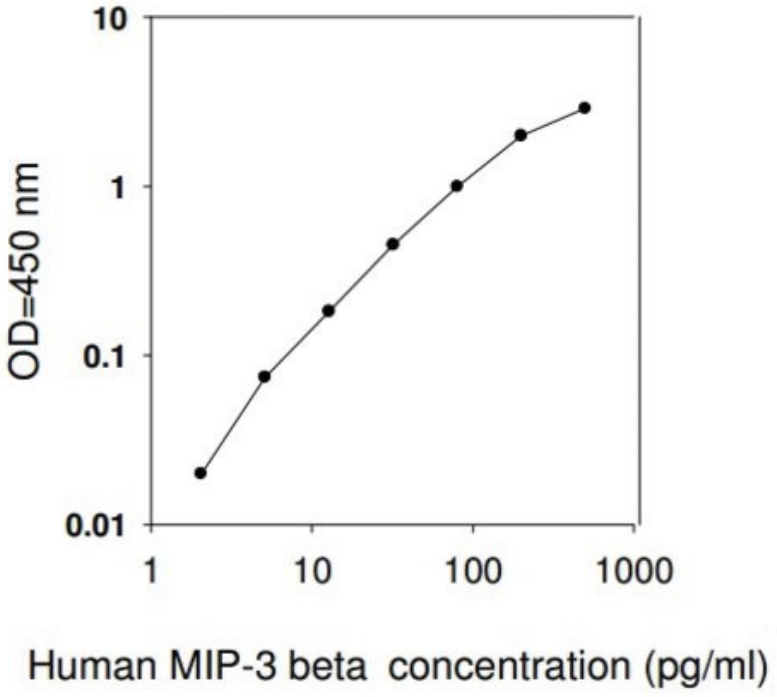
Calculation

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve with relative software which can perform four-parameter logistic regression models, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.

Typical Standard Curve

Preparation of Standard for Human MIP-3 β/CCL19 ELISA Kit, showing dilution factor.





Typical Standard Curve for Human MIP-3 β/CCL19 ELISA Kit. The standard curve(s) displayed are for demonstration only. A standard curve must be run with each assay.

Precautions

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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