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User's Manual

Human CTRP-13 ELISA Kit

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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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This Human CTRP13 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CTRP13 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CTRP13 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CTRP13 samples.

Principles of Testing

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human CTRP13. The capture antibody can bind to the human CTRP13 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human CTRP13 is added to the wells. After another washing of the plate, the streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human CTRP13 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

Reagents And Materials Provided

1. CTRP13 Microplate - 96 well microplate coated with a monoclonal antibody specific for human CTRP13. 1 plate
2. CTRP13 Standard - 1000 ng/vial of lyophilized recombinant human CTRP13. 1 vial
3. Detection Antibody Concentrate - 1.05 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against human CTRP13. 1 vial
4. Positive Control - one vial of lyophilized recombinant human CTRP13. 1 vial
5. Streptavidin-HRP Conjugate - 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate. 1 vial
6. Dilution Buffer - 60 mL of buffered solution with preservative. 1 bottle
7. HRP Diluent Solution - 12 mL of buffered solution with preservative. 1 bottle
8. Antibody Diluent Solution - 12mL of buffered protein based solution with preservatives. 1 bottle
9. Sample Buffer - 20 mL of 0.1% SDS solution. 1 bottle
10. Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative. 1 bottle
11. TMB Substrate Solution - 11 mL of TMB substrate solution. 1 bottle
12. Stop Solution - 11 mL of 0.5M HCl. 1 bottle
13. Plate Sealer, 1 piece
14. Plastic Pouch, 1 piece

Materials Required But Not Supplied

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 - 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.
- 500mM TCEP (fresh preparation)

Storage

Unopened Kit: Store at 2-8°C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened/Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution and TMB Substrate Solution can be stored at 2-8°C for up to 8 months (DO NOT FREEZE and PROTECT FROM LIGHT). All other components may be stored at 2-8°C for up to 8 months.

Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2-8°C after opening.

Specimen Collection And Preparation

SAMPLE COLLECTION AND STORAGE:

Cell Culture Supernates - Centrifuge and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION:

Serum and Plasma samples may require 2-4 fold dilutions.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

Reagent Preparation

Bring all reagents to room temperature before use.

Wash Buffer - Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, warm bottle in a water bath until the crystals

have completely dissolved.

CTRP13 Standard - Reconstitute the CTRP13 standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 1000 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions. Pipette 250 µL of the appropriate Dilution Buffer into tubes #1 to #7. Use the stock solution (1000 ng/mL) to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 ul	1000 ng/ml
1	250 ul of Stock	250 ul	500 ng/ml
2	250 ul of 1	250 ul	250 ng/ml
3	250 ul of 2	250 ul	125 ng/ml
4	250 ul of 3	250 ul	62.5 ng/ml
5	250 ul of 4	250 ul	31.25 ng/ml
6	250 ul of 5	250 ul	15.625 ng/ml
7	250 ul of 6	250 ul	7.813 ng/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note**: This should be prepared 1-2 hours prior to use.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent Solution into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. Note: 1x working solution of streptavidin-HRP should be used within a few days (protect from light). DO NOT FREEZE.

Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer.

Assay Procedure

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL per well of Standard Dilutions in reverse order of serial dilution, sample, or positive control. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature. (Please see plate layout provided.)
5. Aspirate and wash each well with 300 µL of 1x Wash Buffer four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate for 2 hours

on microplate shaker at room temperature.

7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of Anti Rabbit IgG-HRP Conjugate working solution. Cover with plate sealer and incubate for 45 minutes on microplate shaker at room temperature. Protect from light.
9. Repeat the aspiration and wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-8 minutes at room temperature. Protect from light.
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450nm.

Calculation

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

Standard (ng/mL)	Corrected (450nm)
Blank	0 (0.094)
7.813	0.038
15.625	0.087
31.25	0.166
62.5	0.331
125	0.529
250	1.242
500	2.314

Positive Control: 30-130 ng/mL

Precision

Intra-assay Precision: 4-6%

Inter-assay Precision: 8-10%

Detection Range

7.8 - 500 ng/mL

Sensitivity

2 ng/mL

Precautions

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

Limitations

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.