



User's Manual

Human MET proto-oncogene, receptor tyrosine kinase ELISA Kit



DEIA7180



96T



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

For quantitative detection of human C-MET in cell culture supernates, serum and plasma (heparin, EDTA).

General Description

C-Met (MET or MNNG HOS Transforming gene) is a proto-oncogene that encodes a protein known as hepatocyte growth factor receptor (HGFR). MET proto-oncogene has a total length of 125, 982 bp, and it is located in the 7q31 locus of chromosome 7. MET is a membrane receptor that is essential for embryonic development and wound healing. Activation of MET triggers mitogenesis, and morphogenesis.

Principles of Testing

This human C-MET ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for C-MET has been precoated onto 96-well plates. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for C-MET is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human C-MET amount of sample captured in plate.

Reagents And Materials Provided

Catalog number	Description	Quantity
-	96-well plate precoated with anti- human C-MET antibody	1
ST0000-10	Lyophilized recombinant human C-MET standard	10ng/tube×2
AR1107	Biotinylated anti- human C-MET antibody	130µl(dilution 1:100)
AR1103	Avidin-Biotin-Peroxidase Complex (ABC)	130µl(dilution 1:100)
AR1106-1	Sample diluent buffer	30 ml
AR1106-2	Antibody diluent buffer	12ml
AR1106-3	ABC diluent buffer	12ml
AR1104	TMB color developing agent	10ml
AR1105	TMB stop solution	10ml

Materials Required But Not Supplied

- 1 ABC Diluent Buffer, 12ml
- 2 Human C-MET Standard, 10ng * 2
- 3 Sample Diluent Buffer, 30ml
- 4 Antibody Diluent Buffer, 12ml
- 5 TMB Color Developing Agent, 10ml
- 6 Avidin-Biotin-Peroxidase-Complex(ABC 1:100), 130ul
- 7 Biotinylated Anti-Human C-MET Antibody(1:100), 130ul
- 8 TMB Stop Solution, 10ml

9 Coated Microplate

Storage

For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

a) Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

b) Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.

c) Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

a) High target protein concentration (40-400ng/ml). The working dilution is 1:100. i.e. Add 3 µl sample into 297 µl sample diluent buffer.

b) Medium target protein concentration (4-40ng/ml). The working dilution is 1:10. i.e. Add 25 µl sample into 225 µl sample diluent buffer.

c) Low target protein concentration (62.5-4000pg/ml). The working dilution is 1:2. i.e. Add 100 µl sample to 100 µl sample diluent buffer.

d) Very Low target protein concentration (≤ 62.5 pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation

1. Reconstitution of the human C-MET standard: C-MET standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of C-MET standard (10ng per tube) are included in each kit. Use one tube for each experiment.

a. 10,000pg/ml of human C-MET standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 4000pg/ml of human C-MET standard solution: Add 0.4ml of the above 10ng/ml C-MET standard solution into 0.6ml sample diluent buffer and mix thoroughly.

c. 2000pg/ml → 62.5pg/ml of human C-MET standard solutions: Label 6 Eppendorf tubes with

2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 4000pg/ml C-MET standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

2. Preparation of biotinylated anti-human C-MET antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
 - a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
 - b. Biotinylated anti-human C-MET antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1µl Biotinylated anti-human C-MET antibody to 99µl antibody diluent buffer.)
3. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
 - a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
 - b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µl ABC to 99µl ABC diluent buffer.)

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37 °C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard C-MET detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of C-MET amount in samples.

1. Aliquot 0.1ml per well of the 4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml human C-MET standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum or plasma (heparin, EDTA) to each empty well. See "Sample Dilution Guideline" above for details. It is recommended that each human C-MET standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human C-MET antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

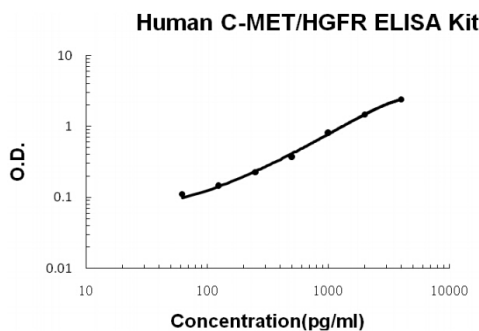


7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
 8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min (Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human C-MET standard solutions; the other wells show no obvious color).
 9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
 10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.
- For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human C-MET concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.



Range 62.5pg/ml-4000pg/ml

Sensitivity < 5pg/ml

Specificity Natural and recombinant human C-MET

Cross-reactivity No detectable cross-reactivity with other relevant proteins

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.

6. Don't reuse tips and tubes to avoid cross contamination.
7. To avoid to use the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre -warmed in 37°C for 30 min before using.

