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hHCCR-1 ELISA Kit

Cat. No. : PY-30001
Target : human HCCR-1

Component	Size	Function
20X capture IgY	500µl	Coated plate with capture IgY
10X dilution	1ml X 4	Dilute IgY, sample with 1X dilution buffer
Standard protein	10µl X 4	Purified recombinant protein (32.768µg/ml)
2X dissolve buffer	100µl	Dissolve recombinant protein
Detection IgY	20µl	Detect IgY antibody (HRP)

Store at 2-8℃. Under these conditions, the kit is stable until the expiration date.

Reagents required, but not supplied

Component	Size	Function
ELISA plate	96 well	Plate not pre-coated with IgY yet
1x PBS buffer	1L	1X Phosphate Buffer Saline
1X PBST buffer	1L	1X Phosphate Buffer Saline 1L with 0.5 ml Tween-20
5% non-fat milk	20ml	100ml 1X PBS buffer with 5g non-fat dry milk
Color development A	100ml	TMB peroxidase substrate www.kpl.com (product code 50-76-01)
Color development B	100ml	Peroxidase substrate solution B www.kpl.com (product code 50-65-00)
Stop solution	100ml	1M H_3PO_4

Test Principle

This kit is based on the sandwich ELISA technique and contains the antibodies for the capture and detection of target protein levels from the sample.

1. Pre-coating the plate with 1X capture IgY.
2. Blocking the plate with 5% Non-fat milk.
3. Incubation of the test sample (serum samples) or standard protein.
4. Detection of antigen with HRP-labeled IgY antibody.
5. Visualization by enzymatic reaction with TMB as a substrate

Reagent Preparations

***Fresh prepare the working solutions as follows. All preparations should be mixed thoroughly and warmed up to the room temperature prior to use.**

1. 1X Dilution buffer: Dilute 10X Dilution buffer with fresh sterile water prior to use.
2. 1X Capture IgY: Dilute the 20X capture IgY with 1X Dilution buffer prior to use
3. ELISA 96 well plate:
   Select the number of coated wells required for the assay.

Blood Collection and Storage

1 · To prepare serum sample, whole blood is directly drawn into a centrifuge tube that contains
· No anti-coagulant. Let blood clot at room temperature for 30 min.
· Promptly centrifuge the blood at 2,000~3,000xg for 15 min at 4℃.
· Transfer and store serum samples in separate tubes. Date and identify each sample.
· Use freshly prepared serum or aliquot and store samples at≦-20℃ for later use. For long term storage, keep at -70℃. Avoid freeze/thaw cycles.
2 · To prepare plasma samples, whole blood should be collected into centrifuge tube containing a final concentration of 1.735 mg/ml K_3EDTA and centrifuged immediately after collection.
3 · If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
4 · Avoid using samples with gross hemolysis or lipemia.

Samples preparation

Dilute 35µl of serum samples with 70µl 1X Dilution buffer (we recommend to use fresh serum for sample preparation).

Standard protein preparation

At a ratio of 1:1, gently mix 2X Dissolve buffer and Purified recombinant protein (32.768µg/ml) for 5-10 minute prior to use, avoid bubbles, till the protein dissolve. The mixture (16.384µg/ml) is not stable over a day.
Standard proteins are prepared by 2 fold serial dilution with 1X Dilution buffer, starting from 256ng/ml. Each well needs 100µl.

Dilution of Detection Antibody conjugated with HRP

Dilute the Detection Antibody at 1:500 with 5% Non-fat milk. Each well needs 100µl.

Substrate Solution:

At a ratio of 1:1, Mix 1 volume of Color Development A and 1 volume of Color Development B prior to use. Each well needs 100µl. The mixture is not stable over a day.

Procedure

1. Application of Standard proteins, samples and controls

1) Pipette 100µl of 1X capture IgY to each well. Cover tightly and incubate at 25°C for 4 hr.
2) Wash the wells of 1 time with 400µl of 1x PBST.
3) Pipette 200µl of 5% Non-fat milk into each well. Cover tightly and incubate at 25°C for 1 hr.
4) Wash the wells of 1 time with 400µl of 1x PBST.
5) Pipette 100µl of prepared serum samples, Standard proteins, and negative controls (fresh prepared 1 X dilution buffer only as a blank) in duplicate to each well. See the pipetting scheme.
6) Cover tightly and Incubate overnight at 4°C.

2. Application of Detection Antibody

1) Wash the wells 1 time with 400µl of 1x PBST.
2) Pipette 100µl of 1:500 diluted Detection IgY Ab (HRP) into each well.
3) Cover tightly and incubate at 25°C for 1 hr.

3. Substrate Reaction

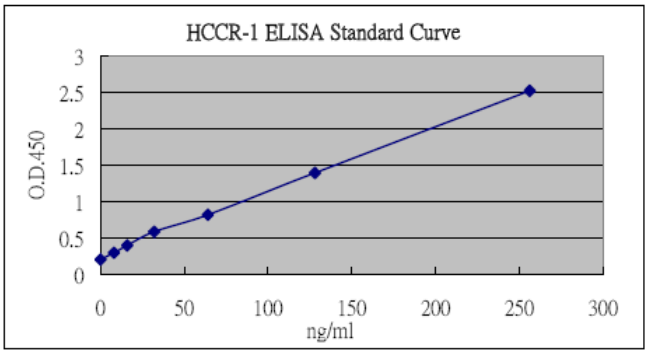
1) Wash the wells 3 times with 400µl of 1x PBST.
2) Apply 100µl of fresh prepared Substrate Solution into each well.
3) Incubate 5 to 20 min at room temperature and add 100µl of Stop Solution into each well.
4) Measure samples against Substrate Solution in a microplate reader at 450nm in 20min.

4. Evaluation of Results

The standard curve is used to determine the amount of target protein in an unknown sample. The standard curve is generated by plotting the average O.D. (450nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding target protein concentration (ng/ml) on the horizontal (X) axis.

1. At first, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the value of a negative control (0 ng/ml: BLANK) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of target protein in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding target protein concentration. If samples generate values higher than the highest concentration, dilute the samples and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.
3. To determine the final concentration of target protein in serum, the concentration read from the standard curve must be multiplied by the dilution factor. In this HCCR-1 kit is 3.

Standard Curve



Typical standard curve, not to be used to calculate data.

ELISA kit protocol

Add 100µl of 1X capture IgY to each well
↓
Incubate at 25℃ for 4 hr
↓
Wash plate of 1 time with 400µl of PBST
↓
Add 200µl of 5% non-fat milk to each well
↓
Incubate at 25℃ for 1 hr
↓
Wash plate of 1 time with 400µl of PBST
↓
Add 100µl of prepared samples, standards and negative control (1X dilution buffer) in duplicate to each well
↓
Incubate overnight at 4℃
↓
Wash plate of 1 time with 400µl of PBST
↓
Add 100µl of diluted detection IgY Ab(HRP) into each well
↓
Incubate at 25℃ for 1 hr
↓
Wash plate of 3 times with 400µl of PBST
↓
Add 100µl of fresh prepared substrate solution into each well
↓
Incubate 5-20min at room temperature
↓
Add 100µl stop solution into each well
↓
Measure samples at 450nm in 20min