Mouse Histidine-rich Glycoprotein(HRG) ELISA Kit

#E-302

Catalog Number: E-302

Detection Range:31.2 pg/mL -2000 pg/mL.

Synonyms:HPRG,Histidine-proline-rich glycoprotein

Principle:

The microtiter plate provided in this kit has been pre-coated with an antibody specific to HPRG. Standards or samples are then added to the appropriate microtiter plate Wells and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain HPRG,enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of HPRG in the samples is then determined by comparing the O.D. of the samples to the standard curve

Materials and components

Reagent	Quantity
Assay plate	1 (12×8wells)
Standard(1600pg/ml)	1 × 0.5ml
Sample Diluent	1 × 6ml
Standard Diluent	1 × 1.5ml
Detection Reagent A	1 ×6ml
Detection Reagent B	1 ×6m;
Wash Buffer(30x concentrate)	1 × 20ml
Substrate	1 × 6ml
Stop Solution	1 × 6ml
Plate sealer	2
Instruction	1

Storage/Stability:

Store at 4℃/6Months

Note for Sample:

1. Samples to be used within 5 days may be stored at 2-8℃ , otherwise samples must stored at -20℃ (1 month) or -80℃ (2 months) to avoid loss of bioactivity and contamination.
2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

Reagent preparation

Standard - Reconstitute the Standard with Standard Diluent

800pg/ml	Standard No.5	150ul Standard with 150ul Standard Diluent
400pg/ml	Standard No.4	150ul StandardNo.5 with 150ul Standard Diluent
200pg/ml	Standard No.3	150ul StandardNo.4 with 150ul Standard Diluent
100pg/ml	Standard No.2	150ul StandardNo.3 with 150ul Standard Diluent
50pg/ml	Standard No.1	150ul StandardNo.2 with 150ul Standard Diluent

Wash Buffer: Dilute 20 mL of WashBuffer Concentrate into deionized or distilled water to prepare 600 mL of Wash Buffer

Assay procedure

1. Add 50 µl of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 30minutes at 37℃.
2. Aspirate each well and wash, repeating the process five times for a total of five washes. Wash by filling each well with Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. and let it sit for 30seconds. 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
3. Add 50 µl of Substrate Solution to each well. Cover with a new Plate sealer.Incubate for30 minutes at 37℃
4. Aspirate each well and wash, repeating the process five times for a total of five washes.
5. Add 50 µl of Detection Reagent A solution to each well,and then 50 µl of Detection Reagent B, Oscillation mixing . Incubate for 10minutes at 37℃.
6. Add 50 µl of Stop Solution to each well.
7. Determine the optical density of each well at once, using a microplate reader set to 450 nm

NOTES

1. Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37℃ directly.).
2. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments