for research use only

Human Nuclear factor-E2 related factor 2 (Nrf2) ELISA Kit

Cat.No: : AAA23628

Storage：2-8°C.

validity： six months.

The sensitivity of this assay is 10 pg/mL

Standard concentration was followed by: 2400、1200、600、300、150、75 pg/mL

Both intra-assay CV and inter-assay CV are less than 15%.

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Intended use

This NRF2 ELISA kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of NRF2 in the sample, this NRF2 ELISA Kit includes a set of calibration standards. The calibration standards are assayed simultaneously with the samples and allow the operator to produce a standard curve of Optical Density versus NRF2 concentration. The concentration of NRF2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Sample collection and storage

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Cell culture supernate and other biological fluids

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles

Note: The samples should be centrifuged adequately and no hemolysis or granule was allowed.

Materials required but not supplied

1. Standard microplate reader (450nm).
2. Precision pipettes and Disposable pipette tips.
3. 37 °C incubator.

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate, and microplates are matched for optimal performance. Use only the reagents supplied by the manufacturer.
2. Do not remove the microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
3. Mix all reagents before using.

Remove all kit reagents from the refrigerator and allow them to reach room temperature (20-25°C).

Materials supplied

Name	96 determinations	48 determinations
Microelisa stripplate	12*8strips	12*4strips
Standard	0.3ml*6 vials	0.3ml*6 vials
Sample diluent	6.0ml*1vial	3.0ml*1vial
HRP-Conjugate reagent	10.0ml*1vial	5.0ml*1vial
20X Wash solution	25ml*1vial	15ml*1vial
Chromogen Solution A	6.0ml*1vial	3.0ml*1vial
Chromogen Solution B	6.0ml*1vial	3.0ml*1vial
Stop Solution	6.0ml*1vial	3.0ml*1vial
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Reagent preparation

20×wash solution: Dilute with Distilled or deionized water 1:20.

Assay procedure

1. Prepare all reagents before starting the assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Add standard: Set Standard wells, and testing sample wells. Add standard 50μl to the standard well.
3. Add Sample: Add 50μl of Standard or Sample to the appropriate wells. Blank wells only add Chromogen Solution A, Chromogen Solution B, and stop solution.

（Dilute the sample appropriately if the sample is not enough, but the test data have to be multiple as the dilution times.）

4. Add 100μl of HRP-conjugate reagent to each well (except blank wells), cover with an adhesive strip, and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400μl) using a squirt bottle, manifold dispenser or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add chromogen solution A 50μl and chromogen solution B 50μl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
7. Add 50μl 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

Calculation of results

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount 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 concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

5. Standard curve