# Hydroxynonenal (HNE) ELISA Kit

Competitive Enzyme Immunoassay for the quantification of Hydroxynonenal (HNE) in cell and tissue lysates, serum, plasma, and purified proteins.

Catalog number: ARG81238

For research use only. Not for use in diagnostic procedures.

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## PRINCIPLE OF THE ASSAY

This is a Competitive Enzyme Immunoassay for the quantification 4-hydroxynonenal (HNE) protein adducts in cell and tissue lysates, serum, plasma, and purified proteins.

The HNE conjugate protein would be coated onto a microtiter plate. HNE-BSA standards or samples are then added to the HNE conjugate protein coated ELISA plate. After a brief incubation, an anti-HNE polyclonal antibody is added to bind the HNE-conjugate protein on the plate, HNE in the samples or HNE-BSA in the standard. After washing, any antibody unbounded on the plate would be wash way. Then HRP-conjugated secondary antibody is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of HNE-antibody complex present on the wells. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of 4-hydroxynonenal (HNE) protein adducts in the sample is then determined by comparing the O.D of samples to the standard curve.

HNE-protein adducts in the samples or HNE-BSA in the standards compete with the HNE-coated plate for antibody binding. High HNE adduct content in a sample or high concentration HNE-BSA standard results in less HNE-antibody binding complex on the plate, resulting in a low signal. Conversely, low HNE content in a sample or low concentration HNE-BSA standard result in most antibody binding to the HNE protein on the plate, producing a higher

signal.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| 96-well microplate | 1 strips X 96 wells | 4°C |
| 10X Wash Buffer | 100 ml | 4°C |
| 1000X Anti-HNE Antibody | 10 µl | -20°C |
| 1000X HRP-Conjugated Secondary Antibody | 20 µl | 4°C |
| Assay Diluent | 50 ml (Ready-to-use) | 4°C |
| TMB substrate | 12ml (Ready-to-use) | 4°C (Protect from light) |
| STOP solution | 12ml (Ready-to-use) | 4°C |
| HNE-BSA Standard (1 mg/ml) | 250 µl | -20°C |
| HNE Conjugate (1 mg/ml) | 50 µl | -20°C |
| 100X Conjugate Diluent | 300 µl | -20°C |

Upon receipt, aliquot and store the Anti-HNE Antibody, HNE-BSA Standard, HNE Conjugate and 100X Conjugate Diluent at -20°C to avoid multiple freeze/thaw cycles. Store all other kit components at 4°C

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water

- 1X PBS
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HNE-BSA Standard, Anti-HNE Antibody and HRP-Conjugated Secondary Antibody before use.
- If crystals are observed in the 10X Wash buffer, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Prepare and mix all reagents thoroughly before use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Note: Haemolytic and especially lipemic samples should not be used with this assay.

## REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. Mix well and storage at 2-8°C.
- **Anti-HNE Antibody and Secondary Antibody:** Dilute the antibodies immediately before use, dilute the 1000X Anti-HNE antibody and 1000X Secondary Antibody into Assay Diluent to yield 1X antibody working solution. Do not store diluted solutions.
- **1X Conjugate Diluent:** Dilute reagent immediately before use, dilute the 100X Conjugate Diluent into 1X PBS to yield 1X Conjugate Diluent. (e.g. Add 50 µl of 100X Conjugate Diluent to 4.95 ml of 1X PBS)
- **10 µg/ml HNE Conjugate:** Dilute reagent immediately before use, dilute the 1.0 mg/ml HNE Conjugate into 1X PBS to yield HNE Conjugate working solution at a concentration of 10 µg/ml. (e.g. Example: Add 25 µl 1.0 mg/ml HNE Conjugate to 2.475 mL of 1X PBS)
- **HNE-BSA standard:** Prepare a series dilution of HNE-BSA standards with Assay Diluent. The Assay Diluent serves as zero standard (0 µg/ml) to determine background, and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

| Standard No | HNE-BSA concentration (µg/ml) | Volume of Assay Diluent (µl) | Volume of standard (µl) |
|---|---|---|---|
| S1 | 200 | 320 | 80 (1 mg/ml stock) |
| S2 | 100 | 200 | 200 (S1) |
| S3 | 50 | 200 | 200 (S2) |
| S4 | 25 | 200 | 200 (S3) |
| S5 | 12.5 | 200 | 200 (S4) |
| S6 | 6.25 | 200 | 200 (S5) |
| S7 | 3.125 | 200 | 200 (S6) |
| S8 | 1.56 | 200 | 200 (S7) |
| S0 | 0 | 200 | 0 |

Note: Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

- **Sample**: If the initial assay found samples contain HNE higher than the highest standard, the samples can be diluted with 0.1% BSA containing 1X PBS and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

## ASSAY PROCEDURE

### HNE Conjugate Protein Coated Plate:

Note: The HNE-Conjugated Protein coated wells are not stable and should be used within 24 hrs after coating. Please only coat the number of wells to be used immediately.

1. Mix 10 µg/ml of HNE Conjugate and 1X Conjugate Diluent at 1:1 ratio as HNE-Conjugated protein coating solution.
2. Add 100 µl of the mixture from step1 to each well and incubate overnight at 4°C.
3. Remove the HNE Conjugate coating solution and wash twice with 1X PBS.

After the last wash, remove any remaining 1X PBS by aspirating, decanting or blotting against clean paper towels.

4. Add 200 µl of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.

## 4-hydroxynonenal (HNE) ELISA procedure

1. Standards and samples should be assayed in duplicates.
2. Remove the Assay Diluent in the wells immediately before use.
3. Add 50 µl of the HNE-BSA standards and samples into the appropriate wells of the HNE-Conjugated protein coated plate. Incubate for 10 minutes at room temperature on an orbital shaker.
4. Add 50 µl of the 1:1000 diluted anti-HNE antibody to each well, incubate for 1 hour at room temperature on an orbital shaker
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of the diluted HRP-Conjugated Secondary Antibody to all wells and incubate for 1 hour at room temperature on an orbital shaker.
7. Aspirate each well and wash as step 5.
8. Warm TMB substrate solution to room temperature. Add 100 µl of TMB substrate solution into each well. Incubate for 2-20 mins at RT on an orbital shaker. Avoid exposure to light.

*Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*

9. Add 100 µl of Stop Solution to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at 450nm (optional: read at 620 nm as reference wave length).

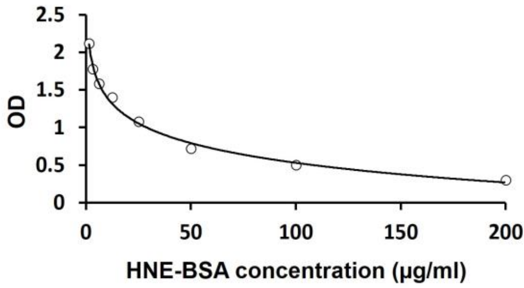
## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using lg-log, semi log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of

data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

1.56 μg/ml

### Assay Range

1.56 - 200 μg/ml