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# ab238538
# Lipid Peroxidation (4-HNE) Assay Kit

For the quantitative measurement of 4-HNE in samples such as purified protein, plasma, serum, and tissue and cell lysates.

This product is for research use only and is not intended for diagnostic use.

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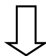
# 1. Overview

Lipid Peroxidation (4-HNE) Assay Kit (ab238538) is designed for the rapid detection and quantitation of 4-hydroxynonenal (4-HNE)-protein adducts.

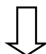
4-HNE is a well known by-product of lipid peroxidation and is widely accepted as a stable marker for oxidative stress. This kit will allow the quantitation of 4-HNE adduct in protein samples determined by comparing its absorbance with that of a known 4-HNE-BSA standard curve.

## 2. Protocol Summary

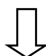
Prepare all reagents, samples, and standards as instructed.



Add 50 µL standard or sample to wells of 4-HNE Conjugate coated plate and incubate for 10 mins. Add 50 µL of the diluted anti-4-HNE antibody and incubate for 1 h.

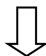


Washing steps with 250 µL 1X Wash Buffer.



Add 100 µL diluted Secondary Antibody-HRP Conjugate per well and incubate for 1 h. Wash as before with 1X Wash buffer.

Add 100 µL of warm Substrate Solution and incubate for 2-20 mins.



Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

## 3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit  datasheet on our website.

## 4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1

year from receipt, if components have not been reconstituted.

– Aliquot components in working volumes before storing at the recommended temperature.
– Avoid repeated freeze-thaws of reagents.

| Item | Quantity | | Storage condition |
|---|---|---|---|
| | 96 test | 32 test | |
| Protein Binding Strip Well Plate | 96 well | 32 well | +4°C |
| Anti-4-HNE Antibody (1000X) | 10 µL | 5 µL | -20°C |
| Secondary Antibody, HRP Conjugate (1000X) | 20 µL | 20 µL | +4°C |
| Assay Diluent | 50 mL | 20 mL | +4°C |
| 10X Wash Buffer | 100 mL | 30 mL | +4°C |
| Substrate Solution | 12 mL | 4 mL | +4°C |
| Stop Solution | 12 mL | 4 mL | +4°C |
| 4-HNE-BSA Standard | 250 µL | 100 µL | -20°C |
| 4-HNE Conjugate | 50 µL | 20 µL | -20°C |
| 100X Conjugate Diluent | 300 µL | 100 µL | -20°C |

# 5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

– Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length)

# 6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

## 6.1 4-HNE Conjugate Coated Plate

**Δ Note:** The 4-HNE Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

6.1.1 Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.

6.1.2 Immediately before use, prepare 10 µg/mL of 4-HNE Conjugate by diluting the 1.0 mg/mL 4-HNE Conjugate in 1X PBS. Example: Add 25 µL of 1.0 mg/mL 4-HNE Conjugate to 2.475 mL of 1X PBS and mix well.

6.1.3 Mix the 10 µg/mL of 4-HNE Conjugate and 1X Conjugate Diluent at 1:1 ratio and add 100 µL of the mixture to each well and incubate overnight at 4°C.

6.1.4 Remove the 4-HNE Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid.

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

## 6.2 1X Wash Buffer:

6.2.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water.

6.2.2 Stir to homogeneity.

## 6.3 Anti-4-HNE Antibody and Secondary Antibody

6.3.1 Immediately before use dilute the Anti-4-HNE antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent.

6.3.2 Do not store diluted solutions.

# 7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

**7.1** Prepare a dilution series of 4-HNE-BSA standards in the concentration range of 0 to 200 µg/mL by diluting the 4-HNE-BSA Standard in Assay Diluent as per the table below.

| Standard # | 1 mg/mL 4-HNE-BSA Standard (µL) | Assay Diluent (µL) | 4-HNE-BSA (µg/mL) |
|---|---|---|---|
| 1 | 80 | 320 | 200 |
| 2 | 200 of standard #1 | 200 | 100 |
| 3 | 200 of standard #2 | 200 | 50 |
| 4 | 200 of standard #3 | 200 | 25 |
| 5 | 200 of standard #4 | 200 | 12.5 |
| 6 | 200 of standard #5 | 200 | 6.25 |
| 7 | 200 of standard #6 | 200 | 3.13 |
| 8 | 200 of standard #7 | 200 | 1.56 |
| 9 | 0 | 200 | 0 |

## 8. Sample Preparation

### General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
  - We recommend that you use fresh samples for the most reproducible assay.
  - Samples can be prepared as you would for any ELISA. Briefly, homogenize or sonicate in any lysis buffer (RIPA with SDS is compatible). Centrifuge the samples at 12000g for 10min and harvest the supernatant as your lysate and proceed with the assay.
  - We do not have a recommended amount of tissue to use when preparing lysates, because this will depend on the HNE level of the sample, which will be different for various sample types. Our recommendation is to start with the most concentrated sample possible and prepare further dilutions later, if necessary, after running a small scale sample titration against the standard curve.
  - If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. 4-HNE adducts are very stable and samples can be stored at -80°C for up to six months without 4-HNE degradation. Freeze-thaws should be avoided.

# 9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards and samples in duplicate.

**9.1** Add 50 µL of unknown sample or 4-HNE-BSA standard to the wells of the 4-HNE Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 10 mins on an orbital shaker.

**9.2** Add 50 µL of the diluted anti-4-HNE antibody to each well, incubate at room temperature for 1 h on an orbital shaker.

**9.3** Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

**9.4** Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 h at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 9.3 above.

**9.5** Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 mins on an orbital shaker.

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

**9.6** Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

**9.7** Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

# 10. Data Analysis

- Samples producing signals lower than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

**10.1** Average the duplicate reading for each standard and sample.

10.2 Plot the corrected values for each standard as a function of the final concentration of 4-HNE Adduct.

10.3 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.4 Apply the corrected sample OD reading to the standard curve to get 4-HNE Adduct µg/mL amount in the sample wells.

10.5 Concentration of 4-HNE Adduct in µg/mL in the test samples is calculated as:

$$4 - HNE\ Adduct\ concentration = B * D$$

Where:

B = amount of 4-HNE Adduct in the sample well calculated from standard curve in µg/mL

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

## 11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

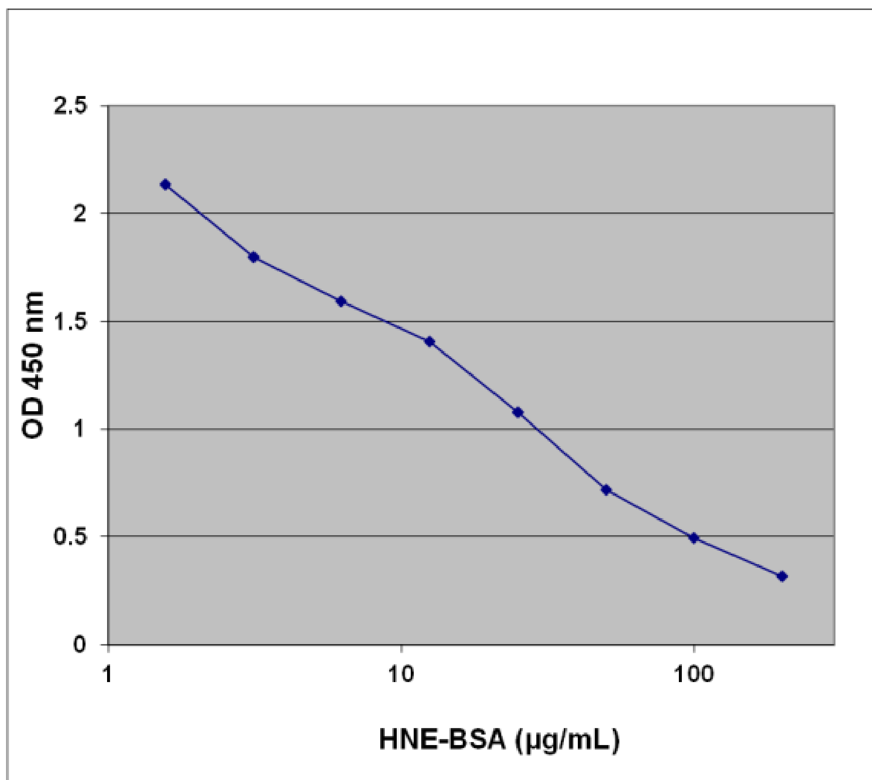



**Figure 1**. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

## 12. Species Reactivity

This kit is not species specific and can be used with samples from any species.

Please contact our Technical Support team for more information.

# 13. Notes

## Technical Support

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