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Life Science Specialties, LLC

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NWLSS™ Hexanoyl-Lysine (HEL) ELISA

Product NWK-HEXL01
For Research Use Only

Simple assay kit for measurement of the lipid peroxidation biomarker Hexanoyl-Lysine adduct in human biological samples such as urine and serum.

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Introduction:

Oxidative damage to lipids (lipid peroxidation) has been found to play an important role in various disease and aging processes. During early stages of lipid peroxidation, lipid hydroperoxides (LOOH) are formed. These can react additionally to form later stage end products such as malondialdehyde (MDA) and hydroxynonenal (HNE). LOOH is sometimes measured to quantify early stage or *acute* lipid peroxidation while MDA is commonly measured to quantify late stage or *chronic* lipid peroxidation. More recently, it has been reported that 13-hydroperoxyoctadecanoic acid (13-HPODE), a precursor to 13-hydroxyoctadecanoic acid (13-HODE) can react with proteins to form measurable adducts by covalently binding to specific amino acid residues. The Hexanoyl-Lysine (HEL) adduct is formed upon oxidative modification of omega-6 fatty acids such as linoleic acid, the predominant polyunsaturated fatty acid (PUFA) in the human diet, and arachidonic acid. HEL may be another useful biomarker for detecting the earlier stages of lipid peroxidation.

Intended Use:

The NWLSS™ Hexanoyl-Lysine ELISA is intended for the quantification of HEL in human biological samples such as urine and serum samples.

Test Principle:

The NWLSS™ Hexanoyl-Lysine ELISA is a competitive enzyme-linked immuno-assay wherein a murine monoclonal antibody to HEL (*Primary Antibody*) and sample or standard are added to a microtiter plate which has been precoated with HEL. Sample or calibrator HEL competes with plate-bound HEL for binding with the antibody. Accordingly, higher concentrations of sample or calibrator leads to reduced binding of the anti-HEL antibody to the HEL coated plate. A subsequent wash step removes free HEL/antibody adduct leaving stationary plate bound HEL complexed to antibody for later detection. Anti-murine IgG antibody conjugated to horse radish peroxidase (*HRP-Conjugate*) is then added to the plate. HRP-conjugate binds to remaining murine anti-8-OHdG and unbound HRP-conjugate is removed in another wash step. Addition of 3,3',5,5' tetramethylbenzidine (*TMB Substrate*) results in blue color development proportional to the amount of anti-HEL antibody bound to the plate and inversely proportional to the concentration HEL in samples or calibrators originally applied to the plate. The reaction is terminated by addition of phosphoric acid (*Stop Solution*) producing yellow color with measurable absorbance at 450 nm.

General Specifications:

Format::	1 X 96 wells	
Number of tests:	Triplicate =	24
	Duplicate =	40
Specificity:	Hexanoyl-Lysine Adduct	
Sensitivity:	LLD = 2 nM in sample applied to plate	

Kit Contents:

Microtiter Plate:	Precoated with HEL	12 X 8 wells
HEL Standard:	Bz-Gly-Hexanoyl-Lys	6 vials (0.5mL)
Primary Antibody:	Mu-Anti-HEL Monoclonal	1 vial (7mL)
Secondary Antibody:	Anti-murine/HRP conjugate	1 vial (Lyoph.)
Secondary Antibody Buffer:	Phosphate buffered saline	1 vial (12mL)
TMB Substrate:	3,3',5,5'tetramethylbenzidine	1 vial (0.25mL)
TMB Buffer:	H_2O_2/Citrate/PBS	1 vial (12mL)
Wash Buffer (5x):	Concentrated PBS	2 vials (25mL)
Stop Solution:	1M Phosphoric acid	1 vial (12mL)
Plate Seal		2 Sheets

Required Materials Not Provided:

Adjustable pipettes capable of transferring 50 µL to 200 µL volumes.
A multi-channel or repeater pipette (recommended).
Reagent Trays (if using multi-channel).
37 °C Incubator
Distilled water

Required Instrumentation:

Microplate reader with 450nm capability.

Warnings, Limitations, Precautions:

Sample/Standard Incubation Temperature

Samples and standards must be incubated overnight at 4 °C. We do not recommend same day incubation at 37 °C.

Possible edge effects

To minimize edge effects, ensure that plate is sealed properly and that incubation times are as uniform as possible. To maintain the most uniform temperature within the wells, it is recommended that any unused wells on a single strip be filled with an equal volume of water prior to incubation.

Storage Instructions:

Upon receipt, store the reagents at 2-8 ºC. This kit is stable if unopened for 12 months from the date of manufacture. Do not use components beyond the expiration date printed on the kit box label.

Unused wells and opened reagents must be stored at 2-8ºC and should be used within two weeks after first opening.

All reagents should be brought to room temperature (18-25ºC) prior to use.

Assay Preparation

Plate Reader Setup

Wavelength:	450 nm
Mode:	Endpoint
Shaker:	On
Curve Fit:	4-Parameter Logistic

Reagent Preparation:

Bring all reagents, plate wells to be used samples and calibrators to room temperature (20-25 °C) before use.

Wash Buffer:

Prepare the necessary volume of wash buffer by mixing 1 part **5X Wash Buffer** with 4 parts distilled water. Label as **Working Wash Buffer.**

Secondary Antibody: (Blue Cap and Label)

Reconstitute the **Secondary Antibody** with **Secondary Antibody Buffer.**

TMB Substrate:

Just prior to use (Day 2), prepare the necessary volume of TMB Substrate; mix 1 part **TMB Substrate** with 100 parts **Dilution Buffer.** Label as **Working TMB Substrate.**

Sample Handling/Preparation:

The multi-disciplinary interest in measuring lipid peroxidation biomarkers has resulted in a myriad of sample types and experimental conditions. It is beyond the scope of this product insert to describe sample processing in detail for each case. However, general guidelines are provided below for representative sample types.

Urine:

If urine contains insoluble material and is cloudy, samples should be clarified by centrifugation. Human samples should be diluted at least 4X with PBS at pH 7.4. Urine samples from experimental animals may need to be diluted 10-20X prior to assay. If urine is known to contain protein, it must be treated according to the serum sample preparation below.

Serum:

1. Prepare a 14mg/mL, pH 7.4 solution of alpha-chymotrypsin in PBS. Label as **Enzyme Reagent.**

2. Dilute serum sample at least 2X with PBS, pH 7.4.

3. Add 60 µL **Enzyme Reagent.** To 300 µL **Diluted Sample** and incubate overnight at 37°C.

4. Filter (Day 2) using ultrafiltration device with molecular weight cutoff of 10kDa (e.g. Millipore, Microcon YM-10 for example) and remove enzymes.

5. Apply the filtrate obtained to the ELISA.

Assay Protocol:

Standard Procedure for Microplate Assay

1. Bring all reagents to room temperature.

2. Remove appropriate number of wells for assay from foil pouch.

3. Construct an assay template to ensure proper sample addition.

4. Add 50µL of **Sample** or **Standard** to each well to be assayed.

5. Add 100µL **Diluted Wash Buffer** to blank wells.

6. Add 50µL **Reconstituted Primary Antibody** to each well except blanks.

7. Shake lightly side to side to ensure proper mixing.

8. Cover plate with adhesive strip, then incubate overnight at 4°C.

9. Empty contents of wells into sink and blot on paper towel to remove as much fluid as possible.

10. Wash plate 3 times as follows:
Add 250µL **Working Wash Buffer** per well
Shake plate slightly during soak period for best wash results.
Empty wash solution into sink by inversion then blot plate against clean paper towel to remove any remaining washing buffer.

11. Add 100µL of **Reconstituted Secondary Antibody** per well.

12. Shake lightly side to side to ensure to mixing.

13. Cover the plate with an adhesive strip then incubate at Room Temp (20 -25°C) for 1 hour.

14. Empty contents of wells into sink and blot on paper towel to remove as much fluid as possible.

15. Wash the plate 3 times as in Step 10.

16. Add 100µL of **Working TMB Substrate** per well.

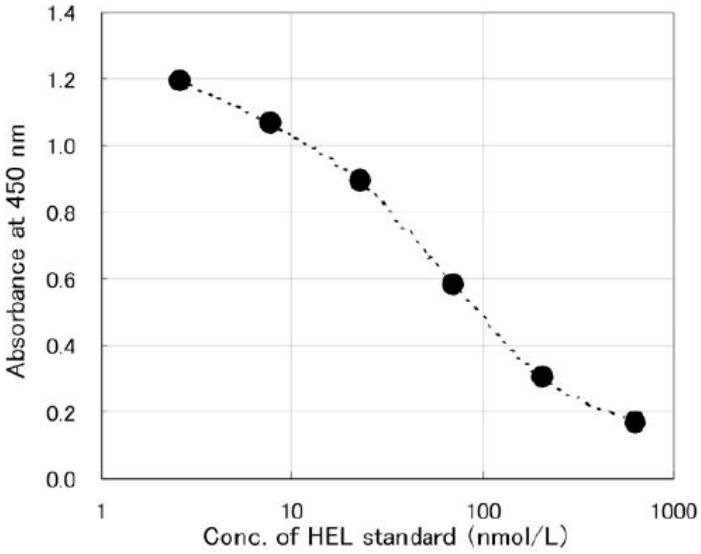
17. Shake lightly side to side to ensure proper mixing.

18. Incubate for 15 minutes, at room temperature in the dark.

19. Add 100µL of **Stop Solution** per well.

20. Measure the absorbance at 450nm.

Data Analysis:
Create a standard curve by plotting Absorbance vs. Concentration (log scale) for each standard level assayed. If available, set the plate reader to utilize 4-Parameter curve fit. An example standard curve is shown below.



References:
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Journal of Biological Chemistry, Vol. 274, No. 29, pp. 20406-20414, (1999).

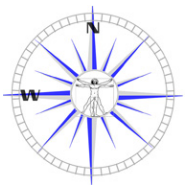
2. Y. Kato, Y. Miyake, K. Yamamoto, Y. Shimomura, H. Ochi, Y. Mori, T. Osawa
Preparation of a monoclonal antibody to N^{ε}-(hexanonyl) lysine: application to the evaluation of protective effects of flavonoid supplementation against exercise induced oxidative stress in rat skeletal muscle.
Biochem. Biophys. Res. Commun. 274(2), p389-393 (2000)

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Notes:

Notes:



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