Hexanoyl-Lysine adduct (HEL) ELISA

Catalog Number: HEL39-K01
96 Wells
For Research Use Only
v. 1.0

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Intended Use:

The Eagle Biosciences Hexanoyl-Lys adduct (HEL) ELISA assay kit is intended for the quantitative determination of Hexanoyl-Lysine adduct in urine, serum or biological samples by enzyme linked immunoassay (ELISA). The Hexanoyl-Lysine adduct ELISA assay kit is for research use only and not to be used in diagnostic procedures.

Assay Background:

Hexanoyl-Lys adduct (HEL) is formed by the reaction of linoleic acid hydroperoxide and Lysine, and a biomarker for oxidative stress. The Hexanoyl-Lysine adduct ELISA assay kit is a competitive enzyme-linked immunosorbent assay for quantitative measurement of hexanoyl-Lys adduct. This kit is based on the monoclonal antibody clone 5H4, which is specific for HEL. Suitable for urine, serum and other biological samples.

Principle of Procedure:

1) Prepare microtiter plate precoated with hexanoy-Lys adduct (HEL).
2) Add HEL standard solution or sample to microtiter plate well, and subsequently add anti HEL monoclonal antibody. The HEL in the standard or sample competes with the HEL on the well surface for the anti HEL antibody. As a result, higher concentration of HEL in sample will result in reduced binding of the antibody bound to the surface of the well.
3) The antibody bound to the HEL in sample, is removed from the well by washing. While the antibody bound to precoated HEL remain on the surface of the well.
4) Peroxidase-conjugated secondary antibody is added to the well, and binds to the anti HEL antibody.
5) Unbound secondary antibody is removed by washing.
6) Addition of the chromatic reagent results in the development of color in proportion to the amount of antibody bound to the well. The reaction is terminated by stop solution. Absorbance at 450 nm is measured using microtiter plate reader.
7) Make a calibration curve from the absorbance data of standards, and calculate the concentration of HEL in the sample.

Materials Provided:

1) HEL Microtiter Plate : Precoated with HEL (8x12 wells, Split Type) 1 Plate
2) Primary Antibody : Monoclonal Antibody specific for HEL 1 Vial (7mL)
3) Secondary Antibody : HRP-Conjugated Anti Mouse IgG Antibody 1 Vial
4) Secondary Antibody Buffer : Phosphate Buffered Saline 1 Vial (12mL)
5) Chromogen : 3,3',5,5'-Tetramethylbenzidine 1 Vial (250 μL)
6) Chromogen Buffer : Hydrogen Peroxide/ Citrate-Phosphate Buffer 1 Vial (12 mL)
7) Washing Buffer (5X) : Concentrated Phosphate Buffered Saline 2 Vials (25mL X 2)
8) Stop Solution : 1M Phosphoric acid 1 Vial (12mL)
9) Standard A-F : Bz-Gly-Hexanoyl-Lys 1 Vial each(500 μL)
   i. (A: 2.6, B:7.7, C:22.7, D:69.7, E:207, F:624 nmol/L)
10) Plate Seal : Adhesive seal to prevent evaporation 2 Sheets

*Storage conditions: Store at 2-8℃. Don't freeze.
*Expiration: After the vials are opened, the kit should be used in one week.
*Measuring range: 2 – 700 nmol/L

Materials required but not provided

- Distilled water (Preparation of washing solution):
- 50 µL micropipettor and pipette tips
- 8-channel (50-200 µL) micropipettor and tips.
- Reagent trays for 8-channel micropipettor.
- 4-7 °C incubator.
- Microtiter plate reader (measuring wavelength 450 nm).

Sample Preparation:

A. Urine sample
Dilution of samples at least 4 times using phosphate buffered saline (PBS at pH 7.4). For urine sample from experimental animal such as dogs and cats, 10 times or 20 times dilution is recommended. If insoluble materials are observed, remove them by centrifugation. If the urine contains proteins, treat the urine by the same procedure as for serum sample.

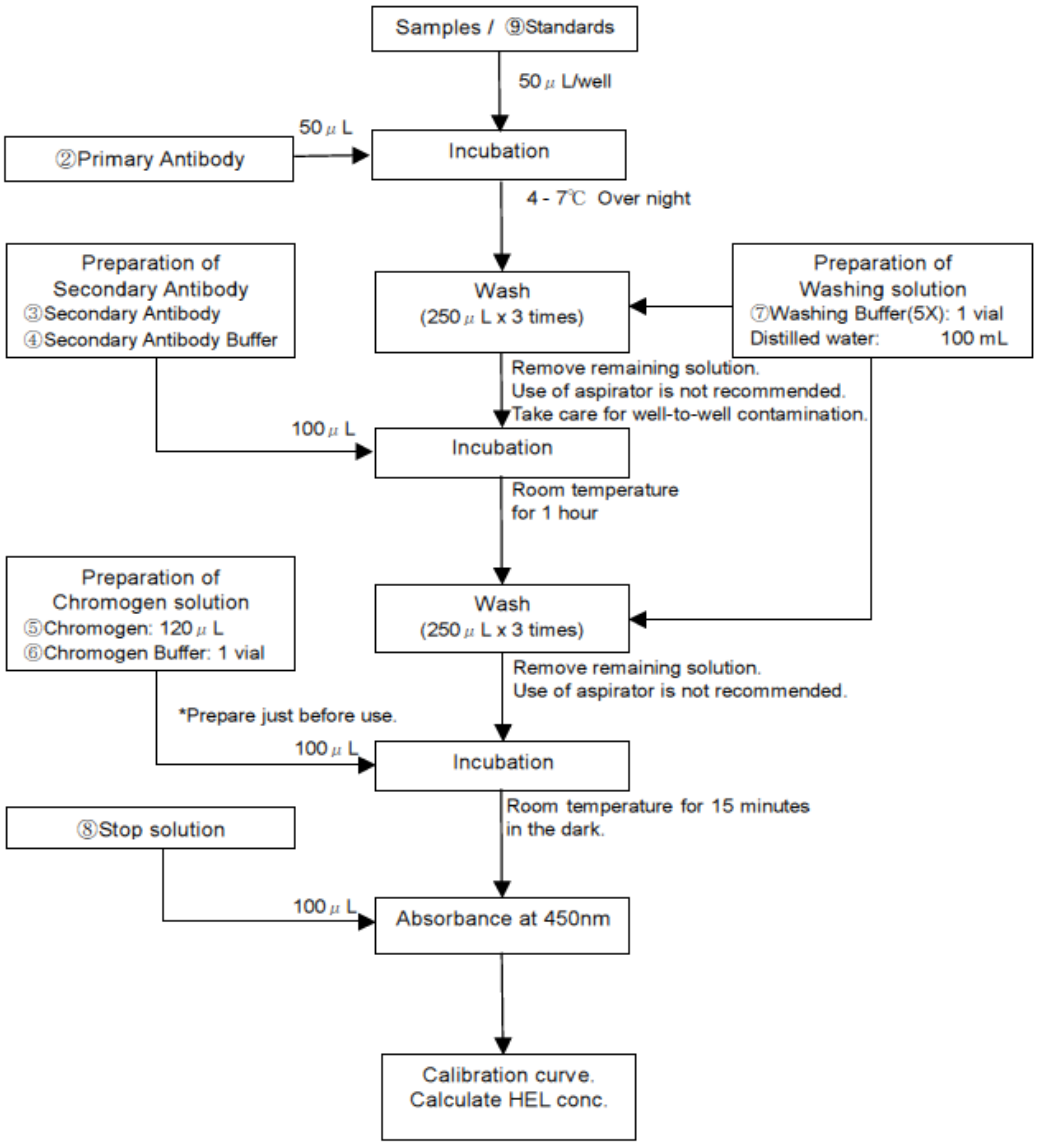
B. Serum sample
1) Prepare "Enzyme reagent", by dissolving 14 mg/mL of alpha-chymotrypsin in PBS (pH7.4).
2) Dilute the serum sample at least 2 times using PBS (pH7.4).
3) Mix 300 µL of diluted sample and 60 µL of "Enzyme reagent". Incubate at 37 °C overnight.
4) Filtrate using ultra filter with cut-off molecular weight 10kDa (for example Microcon YM-10, Millipore), and remove enzymes. Apply the filtrate to ELISA.
*This is an example of procedure. Please investigate optimum condition depending on the sample.

Assay Procedure:

Bring all reagents to room temperature before beginning Hexanoyl-Lys adduct (HEL) ELISA assay kit. Determine the number of microwells needed for the assay (each sample, standard, and control should be assayed in duplicate).

A. Pick out *Microtiter plate* from the bag. To use some wells at the next experiment, remove the well splits from the frame, put it into the bag, and store at 4°C. It will be available for 1 week.
B. Prepare *Washing solution* by mixing one bottle of *Washing buffer(x5)* and 100 mL of distilled water.
C. Add 50 µL of *Standards(A-F)* or sample per well. For the *Blank well*, add 100 µL of *Washing solution*.
D. Add 50 µL of *Primary Antibody* to all wells except *Blank well*. Seal the microtiter plate tightly with *Plate Seal*. Mix gently by shaking the microtiter plate horizontally. Incubate at 4°C overnight.
E. Reconstitute *Secondary Antibody* with one bottle of *Secondary Antibody Buffer*. This is stable for 1 week at 4°C.
F. Remove the plate seal, and pour off the contents of microtiter plate by turning the plate upside down. The use of aspirator is not recommended. Remove the remaining solution by blotting the plate against clean paper towel. Add 250 µL of *Washing solution* to each well, mix gently by horizontal shaking, and remove the contents similarly. Repeat washing procedure twice and remove the remaining solution of the well.
G. Add 100 µL of *Secondary Antibody* to all wells. Seal the microtiter plate tightly with *Plate Seal*. Mix gently by shaking the microtiter plate horizontally. Incubate at room temperature for 1 hour.
H. Prepare *Chromogen solution*. Add 120 µL of *Chromogen* to *Chromogen Buffer* bottle. Please note that Chromogen solution should be prepared just before use. Alternatively, dilute *Chromogen* with 100 volumes of *Chromogen Buffer*.
I. Remove the plate seal, and wash the plate as mentioned at STEP F for 3 times. Remove the remaining solution of the well.
J. Add 100 µL of *Chromogen solution* to all well, and incubate at room temperature for 15 minutes in the dark.
K. Add 100 µL of *Stop Solution* to all wells, mix gently, wait for 3 minutes, and measure the absorbance at 450 nm.

Assay Procedure Flow Chart:



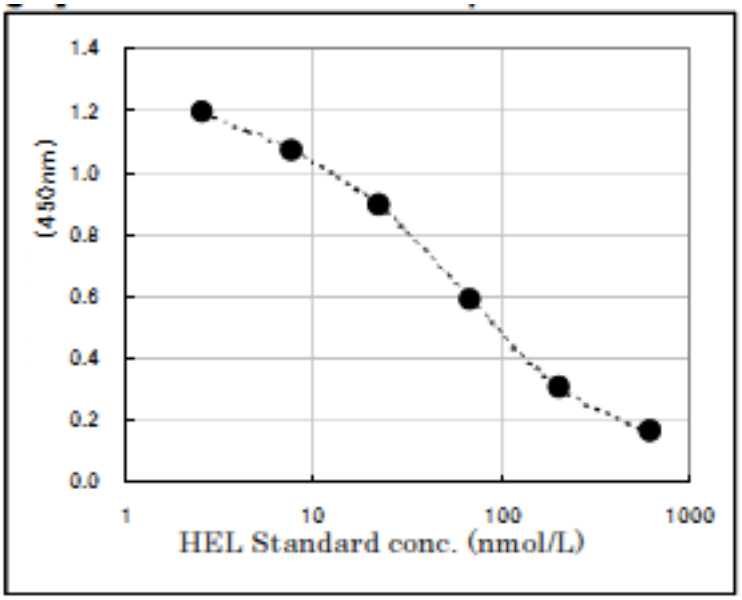
Calculations:

Generate the standard curve of the Hexanoyl-Lys adduct (HEL) ELISA assay kit by plotting absorbance vertical axis and log of concentration as the horizontal axis. An example is shown below. Any smooth curve fit may be applicable. Please note that the standard curve should be established for every assay.

Typical standard curve:

	HEL conc. (nmol/L)	Absorbance 450nm
Standard A	2.6	1.197
Standard B	7.7	1.07
Standard C	22.7	0.895
Standard D	69.7	0.586
Standard D	207	0.305
Standard F	624	0.166

The curve given above is only for demonstration. It must not be used for calculation of samples.



References:

1) Yoji Kato, Yoko Mori, Yuko Makino, Yasujiro Morimitsu, Sadayuki Hiroi, Toshitsugu Ishikawa, Toshihiko Osawa. Formation of N-(hexanonyl) lysine in protein exposed to lipid hydroperoxide. Journal of Biological Chemistry, Vol. 274, No. 29, pp. 20406 – 20414, 1999

2) Yoji Kato, Yoshiaki Miyake, Kanefumi Yamamoto, Yoshiharu Shimomura, Hirotomo Ochi, Yoko Mori, Toshihiko Osawa. Preparation of a monoclonal antibody to N -(hexanonyl) lysine: applycation to the evaluation of protective effects of flavonoid supplementation against exercise-induced oxidative stress in rat skeletal muscle. Biochem. Biophys. Res. Commun., Vol. 274(2), pp389-393, 2000

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