KAMIYA BIOMEDICAL COMPANY

Hexanoyl-Lys adduct (HEL) ELISA

For the quantitative determination of hexanoyl-Lys adduct in urine, serum, and other biological samples.

Cat. No. KT-519

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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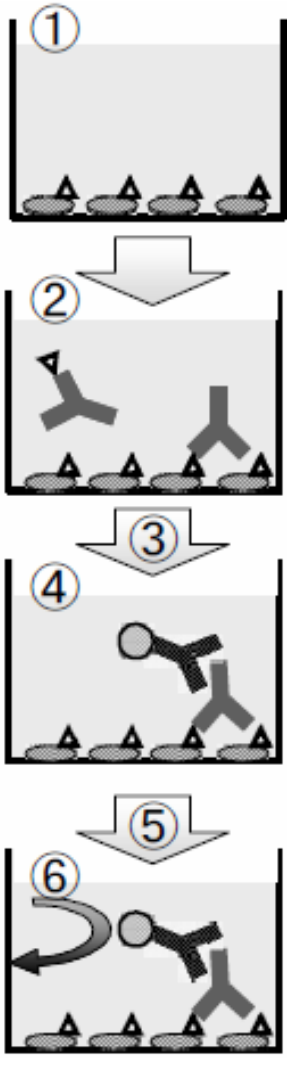
PRODUCT

Hexanoyl-Lys adduct is formed by the reaction of linoleic acid hydroperoxide and Lysine, and is a biomarker for oxidative stress. The ***K-ASSAY®*** Hexanoyl-Lys adduct (HEL) ELISA is a competitive enzyme-linked immunosorbent assay for the quantitative determination of hexanoyl-Lys adduct. Suitable for urine, serum and other biological samples. For research use only. Not for diagnostic nor medical use.

PRINCIPLE

1. Prepare the microtiter plate pre-coated with hexanoyl-Lys adduct (HEL).
2. Add HEL calibrator solution or sample to the microtiter plate well and subsequently add anti-HEL monoclonal antibody. The HEL in the calibrator or sample competes with the HEL on the well surface for the anti-HEL antibody. As a result, higher concentrations of HEL in the sample will result in reduced binding of the antibody bound to the surface of the well.
3. The antibody bound to the HEL in the 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 a microtiter plate reader.
7. Make a calibration curve from the absorbance data of calibrators, and calculate the concentration of HEL in the sample.

Figure 1



COMPONENTS

1. Pre-coated HEL Microtiter Plate: 8 x 12 wells (split type)
2. Primary Antibody: Anti-HEL monoclonal antibody, 7 mL
3. Secondary Antibody: HRP-conjugated anti-Mouse IgG antibody
4. Secondary Antibody Buffer: Phosphate buffered saline, 12 mL
5. Chromogen: 3,3',5,5'-tetramethylbenzidine, 250 µL
6. Chromogen Buffer: Hydrogen peroxide / Citrate-Phosphate Buffer, 12 mL
7. Washing Buffer (5X): concentrated Phosphate Buffered Saline, 2 x 25 mL
8. Stop Solution: 1M Phosphoric acid, 12 mL
9. Calibrators A-F: Bz-Gly-Hexanoyl-Lys (A: 2.6, B: 7.7, C: 22.7, D: 69.7, E: 207, F: 624 nmol/L), 500 µL each
10. Plate Seal: 2 sheets

Materials or Equipment required but not provided

A. Distilled water
B. 50 µL micropipettor and pipette tips
C. 8-channel micropipettor (50-200 µL) and pipette tips
D. Reagent trays for 8-channel micropipettor
E. A 4℃ incubator
F. Microtiter plate reader (measuring wavelength = 450 nm)

PREPARATION OF Samples

A. Urine Sample

Dilute samples at least 4 times using PBS at pH 7.4. For urine samples from experimental animals such as dogs or cats, 10-20 times dilution is recommended. If insoluble materials are observed remove them by centrifugation. If the urine contains proteins, treat the urine using the same procedure as serum samples.

B. Serum Sample

1. Prepare “Enzyme reagent” by dissolving 14 mg/mL of alpha-chymotrypsin in PBS (pH 7.4).
2. Dilute the serum sample at least two times using PBS (pH 7.4).
3. Mix 300 µL of diluted samples and 60 µL of “Enzyme Reagent” and incubate at 37℃ over night.
4. Filter solution using ultra filter with cut-off molecular weight of 10 kDa to remove enzymes. Use the filtrate for the ELISA.

*** This is an example procedure. The optimum conditions depend on the sample and must be determined by the researcher ***

PROCEDURE

1. Bring all reagents, samples and microtiter plate to room temperature before use.
2. Take out the Microtiter plate from the bag. Remove the wells that will not be used from the frame and place them back into the bag and store at 4℃. They will be stable for one week.
3. Prepare the Washing solution by mixing one bottle of 5x Washing buffer with 100 mL of distilled water.
4. Add 50 µL of Calibrators A-F or sample per well. For the Blank well add 100 µL of Washing solution. The typical layout for the microtiter plate is shown in Fig. 2.
5. Add 50 µL of Primary Antibody to all wells except the Blank. Seal the Microtiter plate tightly with the plate seal. Mix gently by shaking the microtiter plate horizontally. Incubate at 4℃ over night.
6. Reconstitute the Secondary Antibody with one bottle of Secondary Antibody Buffer. This is stable for one week at 4℃.
7. Remove the plate seal, pour off the contents of the microtiter plate by turning the plate upside down. The use of an aspirator is not recommended. Remove the remaining solution by blotting the plate against clean paper towels. Add 250 µL of Washing solution to each well, mix gently with horizontal shaking, and remove the contents as before. Repeat washing procedure twice more and remove the remaining solution from the well.
8. Add 100 µL of Secondary antibody to all wells. Seal the microtiter plate tightly with the plate seal. Mix gently by shaking the microtiter plate horizontally. Incubate at room temperature for one hour.
9. Prepare the Chromogen solution. Add 120 µL of Chromogen to the Chromogen Buffer bottle. Please note that the Chromogen solution should be prepared just before use. Alternatively, dilute the Chromogen with 100 volumes of Chromogen Buffer.
10. Remove the plate seal and wash the plate three times as in step 7. Remove the remaining solution from the well.
11. Add 100 µL of the Chromogen solution to all wells and incubate at room temperature for 15 minutes in the dark.

12. Add 100 µL of stop solution to all wells. Mix gently, wait three minutes, and then measure the absorbance at 450 nm.

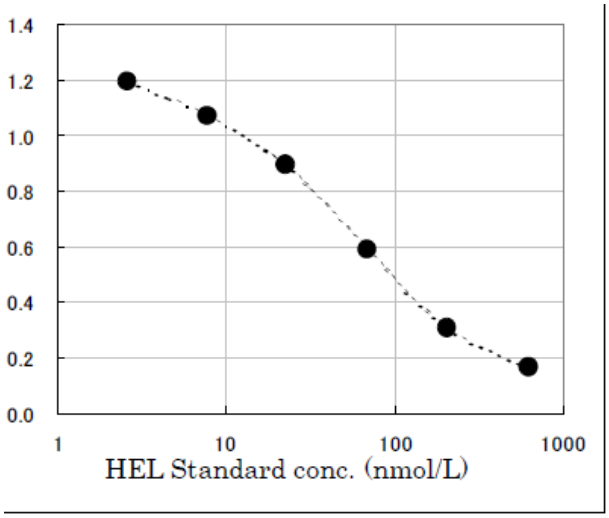
Figure 2 Typical layout of microtiter plate for triplicate assay.
To avoid edge effects, the use of the outmost wells (Rows A and H, marked as "X") is not recommended. 54 wells (18 samples x 3) are applicable for test samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank wells (n=3)			X	X	X	X	X	X	X	X	X
B	Calibrator-A			Sample-1			Sample-7			Sample-13		
C	Calibrator-B			Sample-2			Sample-8			Sample-14		
D	Calibrator-C			Sample-3			Sample-9			Sample-15		
E	Calibrator-D			Sample-4			Sample-10			Sample-16		
F	Calibrator-E			Sample-5			Sample-11			Sample-17		
G	Calibrator-F			Sample-6			Sample-12			Sample-18		
H	X	X	X	X	X	X	X	X	X	X	X	X

CALCULATION OF RESULTS

Generate the calibration curve by plotting the absorbance along the vertical axis and the log of the concentration along the horizontal axis. Any smooth curve fit is applicable. Please note that the calibration curve should be established for every assay.

A typical calibration curve is shown below. Do not use this curve to determine results.



	HEL conc. (nmol/L)	Absorbance(450nm)
⑨HEL Standard-A	2.6	1.197
-B	7.7	1.070
-C	22.7	0.895
-D	69.7	0.586
-E	207	0.305
-F	624	0.166

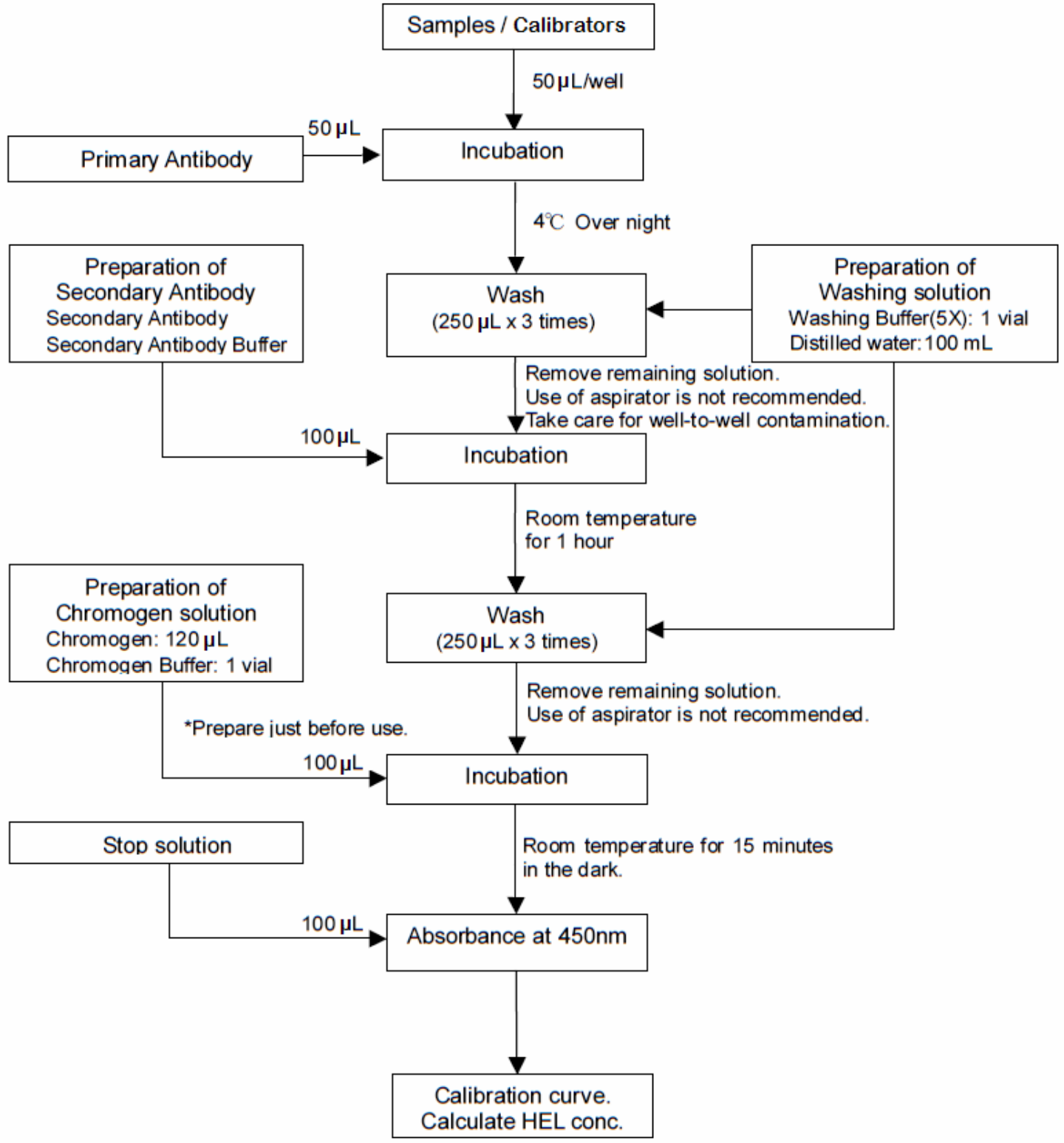
STORAGE

Store at 4℃ until the expiration date. Do not freeze. After the vials are opened, the kit should be used within 1 week.

ASSAY RANGE

2 – 700 nmol/L

Procedure Flow Chart



FOR RESEARCH USE ONLY

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