DATA SHEET

Human HAI-1 ELISA

For the quantitative determination of HAI -1 in human serum, EDTA-plasma or cell culture media.

Cat. No. KT-443

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

Gentaur Molecular Products
Voortstraat 49
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PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Human HAI-1 ELISA is for the quantitative determination of HAI-1 in human serum, EDTA-plasma or cell culture media.

PRINCIPLE
HAI-1 (Hepatocyte growth factor activator inhibitor type1) is one of two types of Kunitz-type serine protease inhibitors that were detected as an inhibitory factor of hepatocyte growth factor activator (HGFA), the factor that converts hepatocyte growth factor/scatter factor (HGF) into the active form. It is said to act as a transmembrane protein having two Kunitz domains, and to inhibit HGFA also as a free form secreted from membrane. HAI-1 also targets various other serine proteases such as matriptase and hepsin. It has been detected in the placenta, kidney, pancreas, prostate, and small intestine, and also reported to express in liver cancer. This kit is designed to measure the free form of Human HAI-1 and is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of human HAI-1.

COMPONENTS
- Microtiter Plate: pre-coated 96-well plate, Anti-Human HAI-1 Mouse IgG mAb affinity-purified
- Labeled antibody: 0.4 mL, (30X) HRP-conjugated Anti-Human HAI-1 Rabbit IgG Fab' affinity-purified
- Calibrator: 2 x 0.5 mL, Human HAI-1
- ELISA buffer: 30 mL, contains 0.05% Tween 20 in PBS with protein stabilizer
- Labeled antibody solution: 12 mL, contains 0.05% Tween 20 in PBS with protein stabilizer
- Chromogen: 15 mL, TMB solution
- Stop solution: 12 mL, 1N H_2SO_4
- Wash buffer: 50 mL, (40X) 0.05% Tween 20 in phosphate buffer

Materials or Equipment required but not provided
- Plate reader (450 nm)
- Micropipette and tip
- Graduated cylinder and beaker
- De-ionized water
- Incubator (37℃ ± 1℃)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of calibrator
- Washing bottle for pre-coated plate
- Disposable test tubes for labeled antibody (30X) and Chromogen: TMB solution

PREPARATION OF REAGENTS
A. Wash Buffer
1. The temperature of the wash buffer should be adjusted to room temperature and then, mix it gently and completely before use.
2. Dilute 50 mL of the wash buffer with 1,950 mL of de-ionized water and mix it. This is the wash buffer for use. This prepared wash buffer should be stored in a refrigerator and used within 2 weeks after dilution.

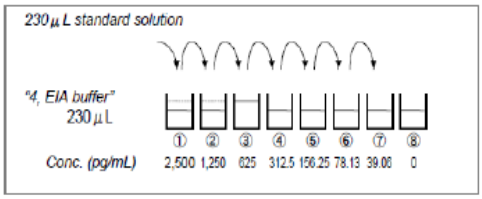
B. Labeled Antibody (30X)

1. Dilute the labeled antibody (30X) with the labeled antibody solution 30 times according to the required quantity into a disposable test tube. Use this resulting solution as the labeled antibody. (Example: For one slit (8-well), the required quantity of labeled antibody is 800 µL, so dilute 30 µL of the labeled antibody (30X) with 870 µL of labeled antibody solution and mix it. Use the resulting solution by dispensing 100 µL into each well). This step should be done just before the application of labeled antibody. The remaining labeled antibody (30X) should be stored at 4℃.

C. Calibrator

1. Put 0.5 mL of de-ionized water into the calibrator vial and mix it gently and completely. This solution is 5,000 pg/mL Human HAI-1 calibrator.
2. To dilute prepare 8 tubes and put 230 µL of the ELISA buffer into the tube.
3. Specify the following concentration of each tube:

    - Tube-1 2,500 pg/mL
    - Tube-2 1,250 pg/mL
    - Tube-3 625 pg/mL
    - Tube-4 312.5 pg/mL
    - Tube-5 156.25 pg/mL
    - Tube-6 78.13 pg/mL
    - Tube-7 39.06 pg/mL
    - Tube-8 0 pg/mL (Test Sample Blank)
4. Put 230 µL of the calibrator solution into tube–1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute the calibrator solution 2 times in series to set up 7 points of diluted calibrators between 2,500 pg/mL and 39.06 pg/mL. Tube-8 is the test sample blank as 0 pg/mL. See figure below.



D. Test Sample

1. Test sample may be diluted with the ELISA buffer if needed. If the concentration of Human HAI-1 in samples cannot be estimated in advance, the pre-assay with several different dilutions is recommended to determine the proper dilution of samples.

PROCEDURE

1. All reagents should be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Calibration curve shall be prepared simultaneously with the measurement of test samples.
2. Determine wells for reagent blank. Put 100 µL of ELISA buffer into the wells.
3. Determine wells for test sample blank, test sample and diluted calibrator.
4. Put 100 µL of test sample blank (tube-8), test sample and dilutions of the calibrator (tube-1~7) into the appropriate wells.
5. Incubate the pre-coated plate for 60 minutes at 37℃ after covering it with plate lid.
6. Wash each well of the pre-coated plate vigorously with wash buffer using a washing bottle. Then, fill each well with wash buffer and leave the pre-coated plate for 15~30 seconds. Remove wash buffer completely from the pre-coated plate by snapping. This procedure must be repeated more than 7 times.
7. Remove the remaining liquid from all wells completely by snapping the pre-coated plate onto paper towel. (If using plate washer, after 4 washes, wash with washing bottle 3 times.)
8. Pipette 100 µL of labeled antibody solution into the wells of the test samples, diluted calibrator and test sample blank.
9. Incubate the pre-coated plate for 30 minutes at 37℃ after covering it with plate lid.
10. Wash the pre-coated plate 9 times in the same manner as before.

11. Take the required quantity of Chromogen and put into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. To avoid contamination, do not return remaining Chromogen into Chromogen bottle.
12. Incubate the pre-coated plate for 30 minutes at room temperature in the dark. The liquid will turn blue with the addition of Chromogen.
13. Pipette 100 µL of the stop solution into the wells. Mix the liquid by tapping the sides of pre-coated plate. The liquid will turn yellow with the addition of the stop solution.
14. Remove any dirt or drops of water on the bottom of the pre-coated plate and confirm there are no bubbles on the surface of the liquid.
15. Run the plate reader and read measurements at 450 nm. The measurement should be done within 30 minutes after the addition of the stop solution.

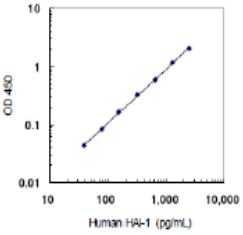
Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1–7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 37°C with plate lid				
Washing 9 times				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm within 30 minutes after application of Stop solution.				

CALCULATION OF RESULTS

1. Subtract the absorbance of test sample blank from all data, including calibrators and unknown samples before plotting.
2. Plot the subtracted absorbance of the calibrators versus the calibrator concentration on log-log graph paper.
3. Draw the best smooth curve through these points to construct the calibration curve.
4. Read the concentration for unknown samples from the calibration curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)
2,500	2.117
1,250	1.195
625	0.634
312.5	0.365
156.25	0.203
78.13	0.125
39.06	0.084
0 (Test Sample Blank)	0.041



*** Note:** A typical calibration curve is shown above. This curve cannot be used to derive test results. Run a calibration curve for each assay.

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PERFORMANCE CHARACTERISTICS

1. Titer Assay

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
10% FCS added RPMI-1640	2	1,232.17	1,250.00	98.6
	4	597.38	625.00	95.6
	8	277.54	312.50	88.8
	16	144.10	156.25	92.2
Human serum	16	1,808.20	1,805.56	100.1
	32	1,002.96	966.44	103.8
	64	512.06	516.39	99.2
Human Plasma (EDTA)	16	1,960.81	2,060.77	95.2
	32	1,092.74	1,119.89	97.6
	64	590.22	612.49	96.4

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640 (x2)	625.00	617.92	98.9
	312.50	278.65	89.2
	156.25	134.98	86.4
Human serum (x16)	2,127.30	2,170.81	102.0
	1,814.80	1,841.35	101.5
	1,658.55	1,624.37	97.9
Human Plasma (EDTA) (x16)	2,116.35	2,130.66	100.7
	1,803.85	1,839.43	102.0
	1,647.60	1,655.36	100.5

3. Intra Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
919.03	63.59	6.9	24
439.21	32.71	7.4	24
72.05	5.74	8.0	24

4. Inter Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
883.80	83.22	9.4	34
422.67	37.84	9.0	34
71.39	5.85	8.2	34

5. Specificity

Compound	Cross Reactivity
Human HAI-1	100.0%
Human HAI-2	≦0.1%
Human HGF	≦0.1%
Human HGFA	≦0.1%
Human c-Met	0.38%

6. Sensitivity : 16.98 pg/mL

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WARNINGS AND PRECAUTIONS

- All reagents should be stored at 4 ℃. All reagents should be brought to room temperature approximately 30 minutes before use.
- The calibrators are lyophilized products. Be careful when opening this vial.
- The stop solution is a strong acidic substance, therefore, be careful not to expose skin and clothes. Dispose appropriately.
- Both the pre-coated plate and the calibrator solution contain sodium azide, therefore dispose of these materials after diluting them with a large quantity of water to avoid the production of explosive metallic azide.
- Precipitation may grow in labeled antibody (30X) however, this will cause no problems in the performance.
- Wash hands after handling reagents.
- Do not mix the reagents with the reagents from a different lot or a different kit.
- Do not use expired reagents.
- Determination of human HAI-1 is affected by the presence of heparin in samples, so please use EDTA-plasma as a sample instead of heparin plasma.

TECHNICAL HINTS

- Test samples should be measured soon after their collection. If test samples must be stored, they should be stored under frozen conditions. Do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measuring.
- The measurements of test samples and the calibrator are recommended to be done in duplicate.
- Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurements.
- Use the wash buffer contained in this kit only for washing the pre-coated plate.
- Insufficient washing may affect the measurements.
- Remove the wash buffer completely by tapping the pre-coated plate on a paper towel.
- Do not wipe wells with paper towel.
- Chromogen should be stored in the dark due to its sensitivity against light. Chromogen should also avoid contact with metals.
- Measurements should be taken within 30 minutes after the addition of the stop solution.

STORAGE

Store at 4℃. The kit is stable as supplied until the expiration date.

FOR RESEARCH USE ONLY

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