KNG1 (Human) ELISA Kit

Catalog Number KA1039

96 assays

Version: 02

Intended for research use only

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Introduction

High molecular-weight kininogen (HK) is a plasma protein coagulation cofactor serving for the activation of zymogens prekallikrein, Factor XII and Factor XI and is also a substrate of each of their proteolytic forms. It circulates as a complex with these zymogens and links the plasma coagulation, fibrinolysis, complement activation, and blood pressure control. HK is produced by the liver and weighs 120 kDa with 626 amino acids. Its plasma concentration ranges from 55 to 90 µg/ml and (1 - 5). HK exhibits anticoagulant properties and is a strong inhibitor of cysteine proteases. Upon cleavage by kallikrein, the released active peptide bradykinin mediates NO release, vasodilation, hypotension and pain. The remaining cleavedHK (HKa) exhibits antiadhesive and antiangiogenic activity, enhancing cell-associated fibrinolysis and releasing cytokines and chemokines to enhance inflammation. Patients with HK deficiency exhibit abnormal surface-mediated activation of fibrinolysis (6 - 7).

Principles of the Test

The KNG1 (Human) ELISA Kit is designed for detection of human Kininogen in urine, saliva, milk, and cell culture supernatant. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Kininogen in less than 4 hours. A polyclonal antibody specific for human Kininogen has been pre-coated onto a 96-well microplate with removable strips. Kininogen in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Kininogen, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Warning and Precautions

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

Reagents

- ✓ **Human Kininogen Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Kininogen.
- ✓ **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- ✓ **Human Kininogen Standard:** Human Kininogen in a buffered protein base (200 ng, lyophilized).

- ✓ **Biotinylated Kininogen Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against Kininogen (80 µl).
- ✓ **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- ✓ **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- ✓ **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- ✓ **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- ✓ **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Instructions

- ✓ Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Opened unused microplate wells may be returned to the foil pouch with the desiccant packets.
- ✓ Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 1 month at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required But Not Provided

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- ✓ **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:200 into MIX Diluent. Store samples at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ **Saliva:** Rinse your mouth at least twice with cool water. Then chew sugarless gum for 1-2 minutes, swallowing saliva as usual. You may continue to chew the gum during saliva collection but please do not spit it into the collection tube. If, for some reason, you are not able to chew the gum, saliva collection is still possible but may take longer. Dilute samples 1:20 into MIX Diluent. The undiluted samples can be stored at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ **Milk:** Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:200 into MIX Diluent. Store samples at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20℃ or below. Avoid repeated freeze-thaw cycles.

Preparation of Reagents

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- ✓ **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8℃.
- ✓ **Standard Curve:** Reconstitute the 200 ng of Kininogen Standard with 2 ml of MIX Diluent to generate a solution of 100 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (100 ng/ml) 1:2 with equal volume of MIX Diluent to produce 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20℃.

Standard Point	Dilution	[Kininogen] (ng/ml)
P1	Standard (100 ng/ml)	100.00
P2	1 part P1 + 1 part MIX Diluent	50.00
P3	1 part P2 + 1 part MIX Diluent	25.00
P4	1 part P3 + 1 part MIX Diluent	12.50
P5	1 part P4 + 1 part MIX Diluent	6.25
P6	1 part P5 +1 part MIX Diluent	3.13
P7	1 part P6 +1 part MIX Diluent	1.56
P8	MIX Diluent	0.00

- ✓ **Biotin Kininogen Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20℃.
- ✓ **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20℃.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30℃).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µl of Kininogen standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 ul of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 time on absorbent paper towel to completely remove the liquid.
5. Add 50 µl of Biotinylated Kininogen Antibody to each well and incubate for one hour.
6. Wash a microplate as described above.

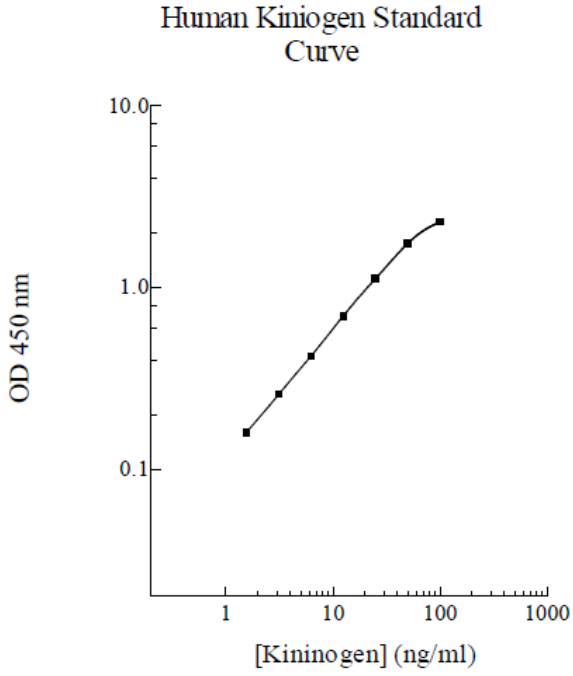
7. Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash a microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- ✓ The minimum detectable dose of Kininogen is typically ~ 1.5 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8% and 7.3% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Milk**	**Saliva**
1:10	100%	95%
1:20	98%	100%
1:40	97%	105%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:100	100%
1:200	105%
1:400	101%

Recovery

Standard Added Value	5 – 50 ng/ml
Recovery %	83-105 %
Average Recovery %	94 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 2
Bovine	None
Monkey	< 10
Mouse	None
Rat	None
Swine	< 2

References

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