Human Kallikrein 3/PSA ELISA Kit

Cat. No. CL0816

96-wells

Target Protein Species	Human
Range	31.2pg/ml-2000pg/ml
Specificity	No detectable cross-reactivity with any other cytokine.

COMPONENTS

Kit Component	Amount
96-well plate precoated with anti-human Kallikrein 3 antibody	1 Plate
Protein Standard: Lyophilized recombinant human Kallikrein 3	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-human Kallikrein 3)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethyllbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

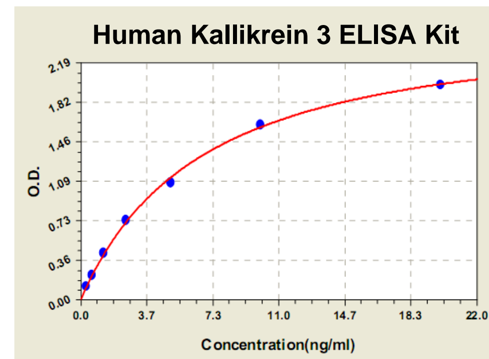
Washing Buffer (not provided): TBS or PBS

0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 900ml H_2O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

0.01M PBS: Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 900ml distilled H_2O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.



X	ng/ml	0.0	0.312	0.625	1.25	2.5	5	10	20
Y	O.D.450	0.069	0.988	1.655	2.457	2.728	3.203	3.231	3.343

Figure 1: Kallikrein 3 Standard Curve. Using the Human Kallikrein 3 ELISA Kit, O.D. data was graphed against Kallikrein 3 protein concentration. The TMB reaction was incubated at 37°C for 15 min.

BACKGROUND

Human tissue kallikrein-related serine peptidases (KLKs) constitute a single family of 15 highly conserved trypsin- or chymotrypsin-like serine proteases encoded by the largest contiguous cluster of protease-encoding genes (KLK1-15) in the human genome mapped to chromosomal locus 19q13.4. The most widely known member of the KLK family is KLK3 or PSA (prostate-specific antigen) that has applications in the diagnosis and monitoring of prostate cancer.[1] Tissue KLKs are usually divided into two groups the “classical” and the “non-classical” KLKs. The term “classical” KLKs is referred to the first members of the human KLK family that were identified, namely KLK1, KLK2, and KLK3 (PSA), whereas the rest are often referred to as “non-classical”. All currently reported KLK genes encode for single-chain prepro-enzymes with lengths varying between 244 and 293 amino acid residues and approximately share 40% protein identity. The preproKLKs are proteolytically processed to enzymatically inactive proKLKs that are secreted via the removal of an amino-terminal signal peptide. Subsequently, proKLKs are activated to mature peptidases extracellularly by specific proteolytic cleavage of their amino-terminal propeptide, a key step in the regulation of KLK functions. Characteristic features of KLKs are the invariant residues of the active-site catalytic triad His57, Asp102 and Ser195, as well as a conserved Gly193 (human chymotrypsin numbering system) which is implicated in stabilizing the oxyanion intermediate of the internal peptide bond during hydrolysis. KLKs are expressed in a wide variety of tissues including the pancreas, heart, lung, central nervous system, salivary glands and endocrine-regulated tissues such as thyroid, breast, testis, ovary, prostate, indicating that they participate in important biological processes. Indeed, several lines of evidence support that KLKs cooperate in complex proteolytic cascade pathways to regulate physiological and pathological processes.[2] For instance, KLK5, KLK7 and KLK14 are involved in skin desquamation and other skin diseases while KLK2, KLK3 and KLK5 have been involved in seminal plasma liquefaction. Of particular note, KLKs are implicated in different stages of cancer development and progression and have emerged as powerful tumor markers as demonstrated by the PSA testing.[3]

References

1. Debela, M. et al: Biol. Chem. 389:623-32, 2008
2. Sotiropoulou, G. et al: J. Biol. Chem. 284:32989-94, 2009
3. Borgoño, C.A. & Diamandis, E.P.: Nat. Rev. Cancer. 4:876-90, 2004

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethyllbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37℃ for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

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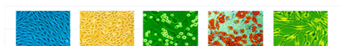

PROTOCOL

I. Plate Washing

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of three washes.

II. Preparation of Test Samples

Test Sample Processing

- **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
- **Serum**: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min.

Sample Dilution Guideline

Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
200-2,000 ng/ml	1:100	1 µl	99 µl
20-200 ng/ml	1:10	10 µl	90 µl
0.312-20 ng/ml	1:2	50 µl	50 µl
≤0.312 ng/ml	n/a	100µl	n/a

If samples will be assayed within 24 hours, store at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

III. Preparation of Reagents

Reconstitution of the Standard

The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.

1. 20ng/ml of human Kallikrein 3 standard solution: Add 0.5 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. 10ng/ml→0.312ng/ml of human Kallikrein 3 standard solutions: Label 6 Eppendorf tubes with 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312 ng/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 20ng/ml Kallikrein 3 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Preparation of Biotinylated Antibody Working Solution

The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

Preparation of the Avidin-Biotin-Peroxidase Complex (ABC) Working Solution

The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

IV. ELISA

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. A standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of target protein amount in samples.

1. Aliquot 0.1ml per well of the 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312 ng/ml human Kallikrein 3 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Blank well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline"** for details. We recommend that each human Kallikrein 3 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human Kallikrein 3 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. （**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.）
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15-20 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human Kallikrein 3 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

V. Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)
- Plot the standard curve:
  Plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The target protein concentration in samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.

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