

Quantikine[®]

Human Kallikrein 3/PSA Immunoassay

Catalog Number DKK300

**For the quantitative determination of human Kallikrein 3/
Prostate Specific Antigen (KLK3/PSA) concentrations in cell
culture supernates, serum, and plasma.**

This package insert must be read in its entirety before using this product.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Human tissue kallikrein 3 (KLK3), commonly known as prostate specific antigen (PSA), is a serine protease of the human tissue kallikrein family (1). It is produced primarily by the epithelial cells lining the prostate gland and secreted into the lumen as an inactive enzyme (proPSA). In seminal plasma, its concentration ranges from 0.5 - 2.0 mg/mL (2). proPSA is a 28 kDa protein with 244 amino acids. By removing 7 amino acids from the N-terminus, it becomes activated and displays chymotrypsin-like enzymatic activity (3). The major physiological function of KLK3 is to cleave the gel-forming proteins in semen, seminogelin I, and seminogelin II, leading to liquefaction of semen clogs after ejaculation (4). KLK3 has also been identified in many other tissues and biological fluids, such as breast and saliva. Its levels in these tissues, however, are over 10^4 times lower than in the prostate and its biological roles in these tissues remain to be elucidated (5).

About 70 - 90% of the KLK3 circulating in serum complexes with α_1 -anti-chymotrypsin (ACT)/serpin A3, with minor amounts binding to α_2 -macroglobulin or α_1 -antitrypsin/serpin A1. The remaining 10 - 30% exists as unbound inactive enzyme (free PSA) (6). When measured with available immunoassays, only free PSA and PSA-ACT are detectable and, traditionally, their sum is termed total PSA. If prostatic tissue damage occurs, such as in prostate cancer and benign hyperplasia, excess amounts of PSA will leak into the circulation, resulting in increased serum total PSA levels. Ratios between total and free PSA are frequently found to be lower in prostate cancer than benign hyperplasia (7).

The expression of KLK3 is regulated by androgen due to the presence of androgen response elements in its gene promoter (8). Measuring KLK3 levels in various biological fluids and cell culture supernates has thus been used to indicate the integrity of the androgen receptor signaling pathway (9, 10).

The Quantikine Human KLK3/PSA Immunoassay is a 4.5 hour solid phase immunoassay designed to measure KLK3/PSA in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human KLK3/PSA, and antibodies raised against the recombinant protein. Natural human KLK3/PSA showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards, indicating that this kit can be used to determine relative levels of natural human KLK3/PSA.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for KLK3/PSA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any KLK3/PSA present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for KLK3/PSA is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of KLK3/PSA bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by enzymes, proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

KLK3/PSA Microplate (Part 892940) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against KLK3/PSA.

KLK3/PSA Conjugate (Part 892941) - 21 mL of polyclonal antibody against KLK3/PSA conjugated to horseradish peroxidase with preservatives.

KLK3/PSA Standard (Part 892942) - 600 ng of recombinant human KLK3/PSA in a buffered protein solution with preservatives; lyophilized.

Assay Diluent RD1W (Part 895117) - 11 mL of a buffered protein solution with preservatives. *For cell culture supernate samples.*

Assay Diluent RD1X (Part 895121) - 11 mL of a buffered protein solution with preservatives. *For serum/plasma samples. May contain a precipitate. Warm to room temperature and mix well before and during use.*

Calibrator Diluent RD5-19 (Part 895344) - 21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*

Calibrator Diluent QD6-5 (Part 895227) - 21 mL of a buffered animal serum with preservatives. *For serum/plasma supernate samples.*

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1W	
	Assay Diluent RD1X	
	Calibrator Diluent RD5-19	
	Calibrator Diluent QD6-5	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution.
- Human KLK3/PSA Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

REAGENT PREPARATION

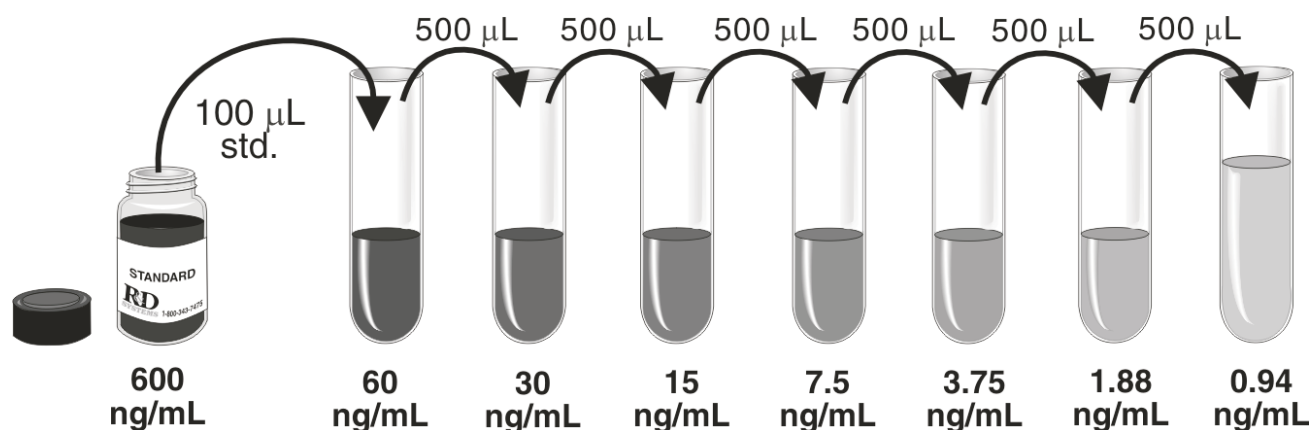
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

KLK3/PSA Standard - Reconstitute the KLK3/PSA Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 600 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of Calibrator Diluent RD5-19 (*for cell culture supernate samples*) or Calibrator Diluent QD6-5 (*for serum/plasma samples*) into the 60 ng/mL tube. Pipette 500 μ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 60 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 ng/mL).



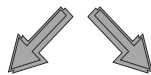
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μL of Assay Diluent RD1W for cell culture samples or Assay Diluent RD1X for serum/plasma samples to each well.
Note: *Assay Diluent RD1X may contain a precipitate. Warm to room temperature and mix well before and during use.*
4. Add 50 μL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 μL of KLK3/PSA Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Serum/Plasma Samples:

Add 100 μ L Assay Diluent RD1X to each well.

Cell Culture Supernate Samples:

Add 100 μ L Assay Diluent RD1W to each well.



3. Add 50 μ L Standard, sample, or control to each well.
Incubate for 2 hours at RT.



4. Aspirate and wash 4 times.



5. Add 200 μ L Conjugate to each well.
Incubate for 2 hours at RT.



6. Aspirate and wash 4 times.



7. Add 200 μ L Substrate Solution to each well.
Incubate for 30 minutes at RT.

Protect from light.



8. Add 50 μ L Stop Solution to each well.
Read at 450 nm within 30 min.
 λ correction 540 or 570 nm

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

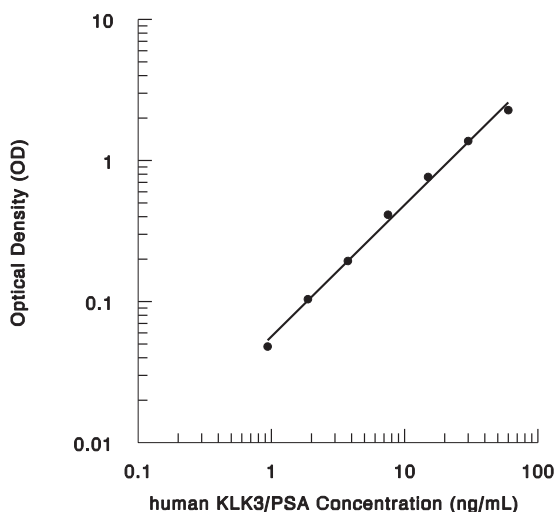
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the KLK3/PSA concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

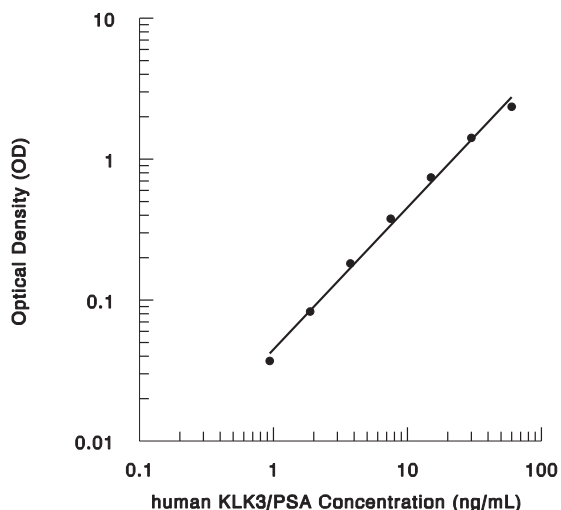
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5-19



ng/mL	O.D.	Average	Corrected
0	0.009 0.010 0.056	0.010	—
0.94	0.060 0.112	0.058	0.048
1.88	0.115 0.203	0.114	0.104
3.75	0.205 0.419	0.204	0.194
7.5	0.426 0.763	0.423	0.413
15	0.787 1.345	0.775	0.765
30	1.422 2.254	1.384	1.374
60	2.318	2.286	2.276

Calibrator Diluent QD6-5



ng/mL	O.D.	Average	Corrected
0	0.007 0.007 0.043	0.007	—
0.94	0.045 0.088	0.044	0.037
1.88	0.091 0.187	0.090	0.083
3.75	0.191 0.373	0.189	0.182
7.5	0.397 0.745	0.385	0.378
15	0.751 1.407	0.748	0.741
30	1.441 2.337	1.424	1.417
60	2.384	2.361	2.354

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	12.3	24.3	44.1	11.8	24.2	43.8
Standard deviation	0.7	1.3	2.0	0.9	0.5	0.8
CV (%)	5.7	5.4	4.6	7.7	5.8	5.5

Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	6.96	15.5	30.0	7.38	16.7	33.0
Standard deviation	0.5	0.7	0.9	0.5	0.9	1.6
CV (%)	7.8	4.5	3.0	6.4	5.3	5.0

RECOVERY

The recovery of KLK3/PSA spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture media (n=4)	99	93 - 105%
Serum (n=4)	102	97 - 109%
EDTA plasma (n=4)	100	93 - 103%
Heparin plasma (n=4)	101	97 - 109%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of KLK3/PSA were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	Heparin plasma (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	110	105	108	106
	Range (%)	105 - 112	103 - 107	105 - 112	104 - 108
1:4	Average % of Expected	110	104	105	109
	Range (%)	104 - 115	97 - 115	101 - 111	104 - 111
1:8	Average % of Expected	106	98	103	99
	Range (%)	104 - 107	95 - 102	94 - 109	95 - 101
1:16	Average % of Expected	95	90	92	90
	Range (%)	89 - 98	85 - 97	85 - 100	85 - 93

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of KLK3/PSA ranged from 0.015 - 0.069 ng/mL. The mean MDD was 0.030 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay utilizes a highly purified NS0-expressed recombinant human KLK3/PSA produced at R&D Systems that is directly calibrated to the NIBSC/WHO 1st International Standard (96/670) for PSA (total).

The NIBSC/WHO 1st International Standard (96/668) for PSA (free) was also evaluated in this immunoassay. This standard parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine KLK3/PSA immunoassay to the approximate NIBSC/WHO 96/668 concentration, use the equation below.

NIBSC/WHO 96/668 approximate value (ng/mL) = 0.505 x Quantikine KLK3/PSA value (ng/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of KLK3/PSA in this assay. No medical histories were available for the donors used in this study.

Sample Type	Range (ng/mL)	% Detectable	Mean of Detectable (ng/mL)
Serum (n=35)	ND - 3.99	8	2.77
EDTA plasma (n=35)	ND - 4.98	8	3.30
Heparin plasma (n=35)	ND - 3.09	8	2.25

ND = Non-detectable

Cell Culture Supernates -

Human peripheral blood cells (1×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural human KLK3/PSA. No detectable levels were observed.

LnCap clone FGC (prostate carcinoma) cells were cultured in RPMI supplemented with 10% fetal calf serum, 10 mM HEPES and 1 nM sodium pyruvate. An aliquot of the cell culture supernate was removed, assayed for levels of natural KLK3/PSA, and measured 25.2 ng/mL.

SPECIFICITY

This assay recognizes recombinant and natural human KLK3/PSA. The factors listed below were prepared at 200 ng/mL in each Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human KLK3/PSA control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Azurocidin/CAP37
C1s
Coagulation Factor 11/Thrombin
Complement component C1r
Enteropeptidase/Enterokinase
Factor D
Granzyme
HGFA
Kininogen (Hka)
KLK1
KLK4
KLK5
KLK7
KLK8
KLK11
KLK13

KLK14
KLKB1
MASP3
Maspin
MPN
Serpina1
Serpina3
Serpina4/Kallistatin
Serpina5
Serpinc1
Serpind1
Serpine1/PAI-1
Serpinf2
Tryptase γ -1/TPSG1
uPA

Recombinant mouse:

Serpinc1
Spinesin

Natural proteins:

α ₂-Macroglobulin

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								