KRISHZYME™ Prekallikrein Activator Assay Kit

REF : KBBA30EP

Ver2.1

(90 test)

RUO

This Kit has been Calibrated against an International Standard from the National Institute of Biologicals and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK.

Chromogenic Assay for estimation of Prekallikrein Activator (PKA) in human blood products and biological preparations according to European Pharmacopeia.

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

REF **KBBA30EP** Σ **90 tests**

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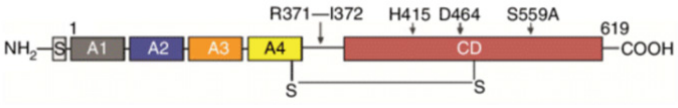
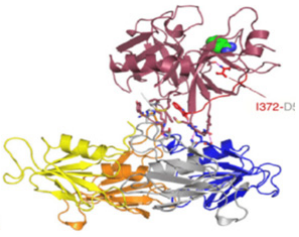
Introduction:

Plasma Prekallikrein (EC 3.4.21.34), is the glycosylated single chain zymogen precursor of the plasma serine protease kallikrein. Kallikrein activates plasminogen in fibrinolysis and cleaves kininogen in the bradykinin system of vasodilation.





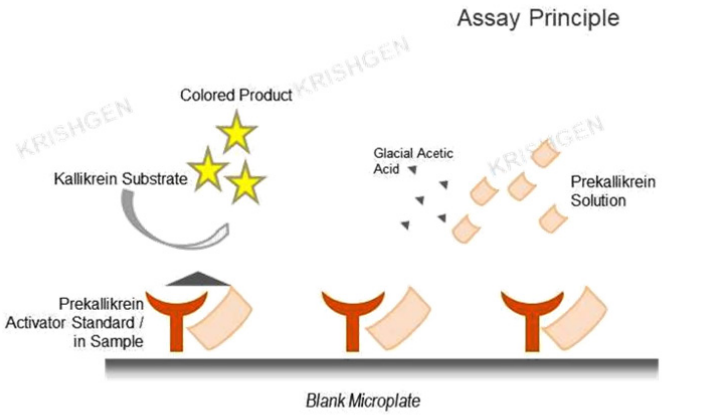
Prekallikrein activator (PKA), also known as activated FXII or activated Hageman F human albumin solutions and immunoglobulin solutions prepared from fractionated plasma. This kit enables the estimation of Prekallikrein activator in such human blood products and biological preparations.

Intended Use:

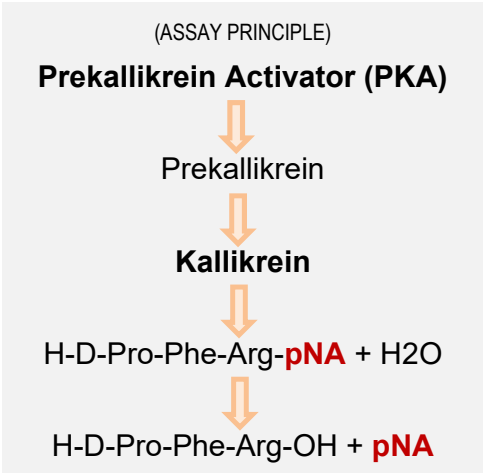
The KRISHZYME™ Plasma Kallikrein Activator Assay Kit utilizes the ability of active plasma kallikrein to cleave a synthetic pNA-based peptide substrate to release pNA, which can be easily quantified using a microplate reader. The kit is easy-to-use and can detect PK activity of purified plasma kallikrein and plasma samples according to the procedure recommended by the European Pharmacopoeia.

Principle:

Plasma Prekallikrein is activated to plasma kallikrein by Prekallikrein activator (PKA -FXIIa). The kallikrein formed releases p-nitroaniline (pNA) from the kallikrein substrate. The rate at which the pNA is released is measured photometrically at 405 nm in a microtitre plate reader. The amount of pNA released is proportional to the amount of PKA present in the preparation up to a concentration of 32 IU/ml. The assay can be performed as rate method as recommended by the European Pharmacopoeia (EP), or by end point. The Human Prekallikrein in the kit is prepared according to the procedure recommended by the European Pharmacopoeia.



PRINCIPLE OF THE KRISHZYME™ PREKALLIKREIN ACTIVATOR ASSAY



Materials Provided:

1. Human Prekallikrein (lyophilized) - 2 vials
2. Kallikrein Substrate (lyophilized) - 2 vials
3. PKA (Prekallikrein Activator) Standard (conc 32 IU/ml, lyophilized) - 1 vial

4. (2X) Buffer A Concentrate - 6 ml
5. Sample/Standard Diluent (lyophilized) - 2 vials
6. Assay Quality Control (lyophilized) - 1 vial
7. Assay Diluent - 1 vial
8. Microtitre Plates (uncoated, blank) - 2 nos
9. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader / Spectrophotometer able to measure absorbance at 405nm
2. Adjustable pipettes to measure volumes ranging from 10 ul to 2500 ul, duly calibrated
3. 1.5 ml Eppendorf tubes
4. Deionized (DI) water
5. Software for data analysis, if required
6. 37°C water bath or dry bath
7. 50% Glacial Acetic Acid
8. Timer/Stop watch
9. Absorbent paper

Reagent Preparation, Storage, and Stability Information:

Unreconstituted (lyophilized) reagents remain stable until the expiration date indicated on the label when stored at 2° to 8°C.

Note:

- Bring all reagents to room temperature.
- All reagents should be diluted immediately prior to use.

1. **Human Prekallikrein**
   Reconstitute with 2.5 ml of distilled water. After reconstitution, store at room temperature (18-24°C) for up to 24 hours before use. For long-term storage, store at -20°C for up to 6 months.
2. **Kallikrein Substrate**
   Reconstitute the vial contents in 1 ml of distilled water. Subsequently, add 9 ml of Buffer B to the reconstituted solution. Post dilution, the diluted substrate remains stable for 8 hours at room temperature, 24 hours at 4°C, and 6 months at -20°C. Be sure to mix well before use.
   Please discard any unused diluted Substrate beyond the indicated storage temperature.
3. **PKA (Prekallikrein Activator) Standard**
   Reconstitute in 1 ml of sample/standard diluent. Allow to stand for 5 minutes, and then mix thoroughly. This reconstituted PKA standard has a concentration of 32 IU/ml.
   Post reconstitution, store at 4°C for up to 8 hours, or freeze at -20°C for up to 6 months
4. **(2X) Buffer A Concentrate:**
   The vial contains 6 ml of 2X concentrated buffer. Prior to use, dilute this 6 ml it by adding 6 ml of sterile distilled water to achieve a final volume of 12 ml. **This is Buffer A.**
   Post dilution store at 4 ºC for up to the kit expiration date.
5. **Buffer B**
   Prepare Buffer B by diluting 1 ml of (2X) **Buffer A** with 9 ml of sterile distilled water. Store at 4°C.
6. **Sample/Standard Diluent**
   Dissolve the contents of the vial in 6 ml of sterile distilled water. Store at 4°C for up to 8 hours, or freeze at -20°C for up to 6 months.

7. Assay Quality Control

Reconstitute the lyophilized Control in 0.5 ml of sterile distilled water. Leave for 5 minutes at room temperature and then mix thoroughly. It is stable for 6 hours at room temperature or 24 hours at 4 °C.

Health Hazard Warnings:

1. The source materials have been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. However adequate care should be taken when handling these materials as a source for potentially infective agents.
2. The Buffer contains Sodium Azide as preservative. It may be harmful if ingested, inhaled or absorbed through the skin. Refer to product MSDS for details.
3. For Research Purpose and In-Vitro Laboratory Use Only.

Specimen Dilution:

Dilute 100 ul of each plasma fraction with 100 ul of Sample/Standard Diluent.

Calibrator Concentration Preparation:

Dilute the reconstituted PKA Standard (32 IU/ml) with Assay Diluent to give PKA concentration values as follows:-

Sr No.	PKA Concentration (IU/ml)	PKA Standard (ul)	Sample/Standard Diluent (ul)
S1	2.0	25	375
S2	4.0	50	350
S3	8.0	100	300
S4	16.0	100	100

Assay Protocol:

Particulars	Tubes
Preparation of Standards and Test Samples -	
Pipette each **PKA prepared Standards** into respective wells.	**25 ul**
Pipette **diluted Test Samples** into respective wells.	**25 ul**
Add **prepared Human Prekallikrein Solution** into respective wells.	**50 ul**
Preparation of Standard Blank and Test Sample Blank -	
Pipette each **PKA prepared Standard** into respective wells.	**25 ul**
Pipette **diluted Test Samples** into respective wells	**25 ul**
Add **Buffer A** [prepared from (2X) Buffer A concentrated] into respective wells	**50 ul**
Transfer the microplate immediately to a plate reader set at 37ºC. Mix well and Incubate for exactly 10 minutes	
Add pre-warmed (37ºC) **diluted Kallikrein Substrate** into all wells.	**100 ul**
Transfer the microplate immediately to a microplate reader set at filter reading of 405nm and 37ºC incubation. Follow the protocol for read as per kinetic assay measurement or end-point assay measurement.	

Particulars	Tubes
For Kinetic Assay Measurement -	
Measure the absorbance change for a total of 5 minutes; starting at 3 minutes through to 8 minutes, depending on your instrumentation and protocols.	in microplate reader
For End-Point Assay	
Incubate the microplate for exactly 5 minutes at 37ºC.	
Stop the reaction by addition of 50% Acetic Acid	**25 ul**
Mix well and measure the absorbance at 405nm.	
Plot the Absorbance subtracted for respective blanks against PKA Concentrations (IU)	

Calculation of Results:

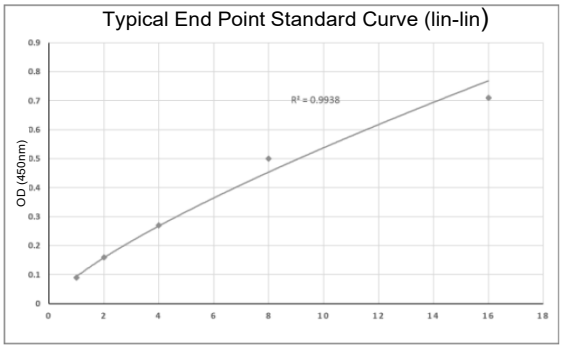
Calculate the Absorbance of each Standard and Test Sample by subtracting the optical densities obtained for the blanks of the Standards and Test Samples from the optical densities obtained for the Standards and Test Samples.

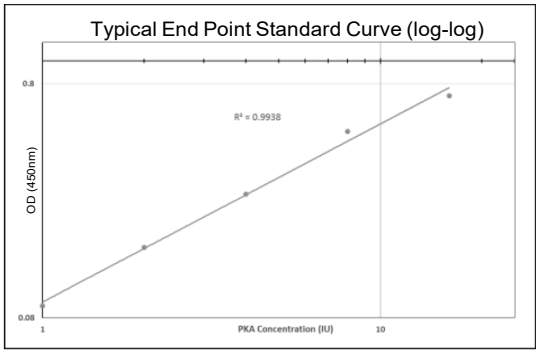
Plot these corrected (blanked) Absorbance of the Standards against PKA Standard Concentrations using a software or linear Graph Paper. Alternately you may also calculate the log Absorbances against the log PKA Standard Concentrations (Log/Log Standard Curve) using a software or log-log Graph Paper.

Calculate the PKA values of the Test Samples from the Standard Curve multiply the values obtained by 2.0 (being the dilution factor of the Test Samples).

Any Test Samples with PKA values greater than 32 IU/ml (or concentration beyond the assay range) must be further diluted with Assay Diluent and re-tested until the absorbance value is obtained within the range of the standard curve. The value then obtained from the standard curve must be multiplied by the total dilution factor to give the actual PKA activity in the test sample.

Typical Standard Curve





Quality Control:
An Assay Quality Control is provided with the kit to verify the validity of the calibration curve and sample results. The expected range of PKA values for this Assay Quality Control is included with the Certificate of Analysis included in this kit.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory.

This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results.

For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Specificity:
This kit has been calibrated against the WHO International Standard, 3rd International Standard For Prekallikrein Activator (NIBSC code: 16/364), National Institute for Biological Sciences and Control, Potters Bar, Hertfordshire, EN6 3QG., UK.
Please note that the calibration is lot specific.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=20 assays) and inter assay (n=20assays) reproducibility on two pools with low, medium, and high concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<15%	<12%
Medium	<10%	<10%
High	<10%	<10%

Standard Range:
2.0 IU/ml - 16 IU/ml.

Recovery by Spiking:
The recovery from Human Albumin solutions spiked with known PKA concentrations (5 to 29IU/ml) yielded an average 98.6% (96-105%) of the theoretical expected value.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

References:

Identification and characterization of prolylcarboxypeptidase as an endothelial cell prekallikrein activator
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- Thrombosis Research, 1982 - Elsevier

Determination of prekallikrein in human plasma: optimal conditions for activating prekallikrein.
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A prealbumin activator of prekallikrein: II. Derivation of activators of prekallikrein from active Hageman factor by digestion with plasmin
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Plasma prekallikrein: isolation, characterization, and mechanism of activation
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- The Journal of Experimental Medicine, 1972 - rupress.org

An improved, reliable and practical kinetic assay for the detection of prekallikrein activator in blood products
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BM Alving, DL Tankersley…
- The Journal of laboratory …, 1983 - translationalres.com

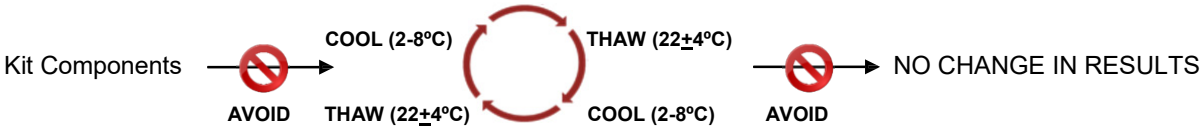
SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

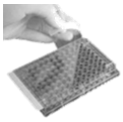
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



PREPARATION OF STANDARDS AND TEST SAMPLES

3.  Pipette **25 ul prepared PKA Standards** and **Test Samples** into respective well.

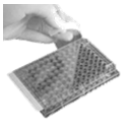
4.  Pipette **50 ul prepared Human Kallikrein solution** into each wells.

5. Transfer plate  to a plate reader and incubate for **exactly 10 mins** at 37°C.

PREPARATION OF STANDARDS AND TEST SAMPLE BLANKS

3.  Pipette **25 ul prepared PKA Standards** and **Test Samples** into respective well.

4.  Pipette **50 ul prepared Buffer A solution** into each wells.

5. Transfer plate  to a plate reader and incubate for **exactly 10 mins** at 37°C.

ASSAY MEASUREMENT

6. For Kinetic Assay measurement, read absorbance at 405nm with a microplate reader  at 37°C for absorbance change for a total of 5 minutes (recommended to start at 3 minutes to 8 minutes).

7. For End-Point Assay, incubate the plate for exactly 5 minutes at 37°C.

8.  Pipette 25 ul of 50% Glacial Acetic Acid to each well to stop the reaction. Mix well.

9. Measure the absorbance at 405nm with a microplate reader. 

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	IU/ml PKA
1A 2A	Assay QC Assay QC			
1B 2B	2 IU/ml 2 IU/ml			
1C 2C	4 IU/ml 4 IU/ml			
1D 2D	8 IU/ml 8 IU/ml			
1E 2E	16 IU/ml 16 IU/ml			
1F 2F	2 IU/ml blank 2 IU/ml blank			
1G 2G	4 IU/ml blank 4 IU/ml blank			
1H 2H	8 IU/ml blank 8 IU/ml blank			
3A 4A	16 IU/ml blank 16 IU/ml blank			
3B 4B	Sample Sample			
3C 4C	Sample Sample			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

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THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Microtiter Plate Blank (12x8 wells)
STD	PKA Standard
Human Prekallikrein	Human Kallikrein concentrated
Kallikrein Substrate	Kallikrein Substrate
2X BUF	(20) Buffer A
STD/SPL DILUENT	Standard / Sample Diluent
ASSY QC	Assay Quality Control
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature