KRISHZYME™ Prekallikrein Activator Assay Kit

REF: KBBA30EP

Ver2.0

(90 test)

RUO

KRISHZYME™ Prekallikrein Activator Assay kit is a chromogenic assay for measuring Prekallikrein Activator (PKA) in human blood products and biologicals 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**

For Research & Industrial Purposes Only. ***NOT*** *for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.*

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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.

Intended Use:

KRISHZYME™ Plasma Kallikrein Activitor 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.

Materials Provided:

1. Human Prekallikrein - 2 vials x 2.5 ml
2. Kallikrein Substrate - 2 vials x 1 ml
3. PKA Standard 32 IU/ml - 1 vial x 1 ml
4. Buffer A Concentrate - 1 vial x 6 ml
5. Sample/Standard Diluent – 1 x 6ml
6. Quality Control – 1 x 0.5 ml
7. Assay Diluent - 1 vial
8. Microtitre Plates - 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. Parallel line 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

Storage and Stability Information:

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

1. Human PreKallikrein

Upon reconstitution, store at room temperature before use for up to 6 hours. For long term storage, the reagent can be stored for 6 months at -20ºC.

2. **Kallikrein Substrate**
   Stability before dilution for 8 hours at room temperature, 48 hours at 4ºC, or at -20ºC for 6 months. Upon reconstitution, store for 6 hours at room temperature or 24 hours at 4ºC

3. **PKA Standard**
   Upon reconstitution, store at 4ºC before use for upto 8 hours, or freeze at -20ºC for 6 months.

4. **Assay Diluent**
   Upon reconstitution, store at room temperature for up to 8 hours or for longer term storage at -20ºC for 6 months.

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.

Reagent Preparation:

Note: 1) Bring all reagents to room temperature.
2) All reagents should be diluted immediately prior to use.

1. **Human Prekallikrein**
   Reconstitute in 2.5 ml sterile distilled water. Mix well before use.

2. **Kallikrein Substrate**
   Reconstitute in 1 ml sterile distilled water and then dilute 1 ml with 9 ml Buffer B before use.

3. **PKA Standard 32 IU/ml**
   Reconstitute in 1.0 ml of sample/standard diluent, leave for 5 minutes at room temperature and mix well. This gives a PKA concentration of 32 IU/ml.

4. **Buffer A Concentrate**
   Tris-HCl buffer (100 mmol/l Tris) containing NaCl (24 mmol/l). Store at 4ºC. The vial contains 6ml of concentrated buffer. Before use dilute the contents of the vial with sterile distilled water to give a final volume of 12ml in the vial. (Buffer A)

5. **Buffer B**
   Dilute 1 ml of Buffer A with 9 ml sterile distilled water.

6. **Assay Diluent**
   Dissolve vial contents in 6 ml sterile distilled water.

Specimen Dilution:

Dilute 100 µl of each plasma fraction with 100 µl of Assay Diluent.

Calibrator Concentration Preparation:

Dilute the PKA Standard with Assay Diluent to give PKA values as follows:-

Sr No.	PKA Concentration (IU/ml)	PKA Standard (µl)	Assay Diluent (µl)
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 PKA prepared Standards into respective microtitre plate wells	25 µl
Pipette diluted test samples into respective microtitre plate wells	25 µl
Add Prekallikrein Solution into respective microtitre plate wells	50 µl
Preparation of Standards and Test Sample Blanks	
Pipette PKA prepared Standard into respective microtitre plate wells	25 µl
Pipette diluted test samples into respective microtitre plate wells	25 µl
Add Buffer A into respective tubes	50 µl
Transfer the microtitre plate immediately to a plate reader set at 37oC. Mix, and incubate for exactly 10 minutes	
Transfer solution from all tubes to respective microwells by pipette in duplicates	25 µl
Add prewarmed at 37ºC, diluted PKA Substrate into microwells	100 µl
Transfer the microplate immediately to a microplate reader set at filter reading of 405nm and 37ºC	
For Kinetic Assay	
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
Plot the Absorbance subtracted for respective blanks against PKA Concentrations (IU)	
For End-Point Assay	
Incubate the microplate for exactly 5 minutes at 37ºC.	
Stop the reaction by addition of 50% Acetic Acid	25 µl
Mix and measure the absorbance at 405nm.	
Plot the Absorbance subtracted for respective blanks against PKA Concentrations (IU)	

Calculation of Results:

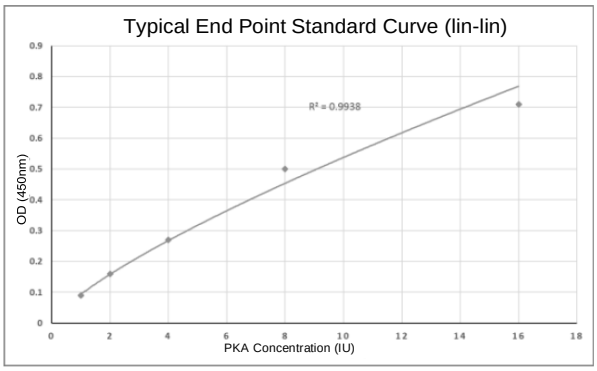
Calculate the Absorbance of each Standard and Test Sample by subtracting the optical densities obtained for the blanks of the Standards and Test Samples from the optical densities obtained for the Standards and Test Samples.

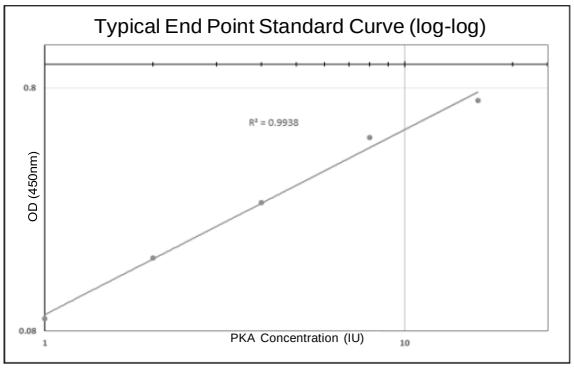
Plot these corrected (blanked) Absorbance of the Standards against PKA Standard Concentrations using a software or linear Graph Paper. Alternately you may also calculate the log Absorbances against the log PKA Standard Concentrations (Log/Log Standard Curve) using a software or log-log Graph Paper.

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 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 Curves as given below:





Performance Characteristics:

Kit Standardization: The assay kit is standardized against the 2nd International Standard for PKA. It is recommended that the PKA high and low positive accuracy controls designed for use with the Prekallikrein Activator (PKA) Assay kit are run with each batch of tests.

Assay Range: 2 – 16 IU/ml

Precision: (n=20)
Intra-Assay CV < 7%
Inter-Assay CV < 9%

Recovery by Spiking:

The recovery from Human Albumin solutions spiked with known PKA concentrations (5 to 29IU/ml) yielded on average 98% (96-105%) of the theoretical expected value.

LIMITED WARRANTY

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