PATHWAY DIAGNOSTICS

PW301EP – 01/2018/Rev

REVISED PROCEDURE
Read new instructions carefully before use

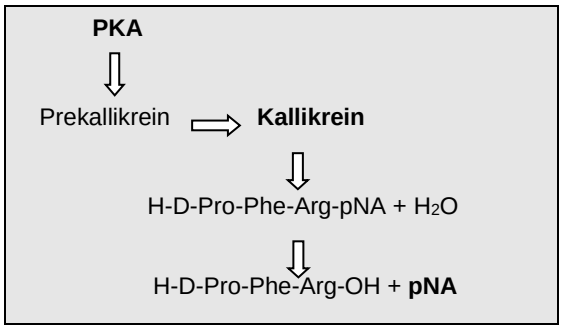
PreKallikrein Activator Assay Kit

An assay kit for the determination of Prekallikrein Activator (PKA) in Human Blood Products and Biologicals according to the European Pharmacopoeia.

PRODUCT CODE: PW301EP 90 tests

For Research Use Only

INTRODUCTION



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

KIT CONTENTS

The kit should be stored at 2-8ºC before use.

1. **Human PreKallikrein (2x 2.5ml)**
   Reconstitute in 2.5 ml sterile distilled water. Store at room temperature before use for up to 6 hours. For longer term storage at -20ºC for 6 months. Mix well before use.
2. **Kallikrein Substrate PW-2302 (2x 1ml)**
   H-D-Pro-Phe-Arg-pNA 3.68 mg/vial plus mannitol. Reconstitute in 1 ml sterile distilled water and then **dilute 1 ml with 9 ml Buffer B (below) before use.** Stability **before dilution**: 8 hours at room temperature, 48 hours at 4ºC, or at -20ºC for 6 months. Stability **after dilution**: 6 hours at room temperature or 24 hours at 4ºC
3. **PKA Standard 32 IU/ml (1x 1ml)**
   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.
   Store this at 4ºC before use for up to 8 hours, or freeze to -20ºC for 6 months.
4. **Buffer A Concentrate (1x6ml)**
   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 6ml of sterile distilled water to give a final volume of 12ml in the vial. (Buffer A)
5. **Buffer B**
   Prepare Buffer B by diluting 1 ml of Buffer A with 9 ml sterile distilled water.
6. **Sample/Standard Diluent (1x 6ml)**
   Dissolve vial contents in 6 ml sterile distilled water. Store at room temperature for up to 8 hours or for longer-term storage at -20ºC for 6 months.
7. **Quality Control (1x 0.5ml)**
   Reconstitute the lyophilised control in 0.5 ml sterile distilled water. Leave for 5 minutes at room temperature then mix thoroughly.
   The reconstituted control is ready to use and does not require dilution.It is stable for 6 hours at room temperature, or 24 hours at 4ºC
8. **Microtitre Plates (x2)**
   The kit is supplied with two, clear plastic 96 well microtitre plates.

STANDARD CURVE

1. **Standard Curve**
   Prepare a serial dilution of the 32 IU/ml PKA standard with standard/sample diluent to give PKA values of 2.0, 4.0, 8.0 and 16.0 IU/ml as follows:

PKA Concentration	PKA Standard	Standard/Sample diluent
IU/ml	µl	µl
2.0	25	375
4.0	50	350
8.0	100	300
16.0	100	100

TEST SAMPLES

Dilute 100 µl of each plasma fraction with 100µl of standard/sample diluent.

ASSAY METHOD

- **Step A for standards and test samples**
  Into microtitre plate wells in duplicate pipette:

  25 µl volumes of each PKA standard dilution or diluted test samples.

  Add 50 µl PreKallikrein solution
- **Step A for standards and test sample blanks**.
  Into microtitre plate wells in duplicate pipette:

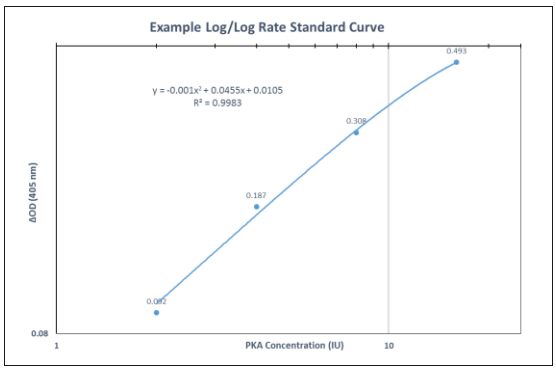
- 25 µl volumes of each PKA standard dilution or diluted test samples.
- Add 50 µl volumes of Buffer A

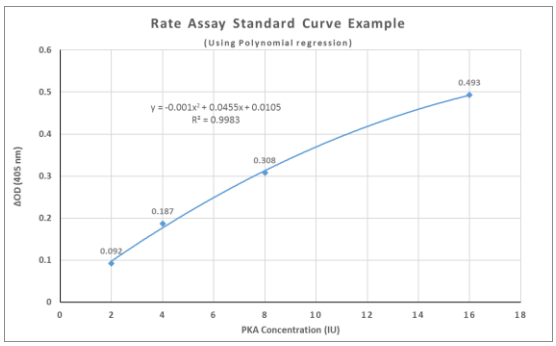
  Transfer the microtitre plate immediately to a plate reader set at 37°C. Mix, and incubate for exactly 10 minutes
- **Step B for standards, test samples and test sample blanks**
  Pre-warm diluted kallikrein substrate at 37°C
  Using a multipipette add 100 µl diluted kallikrein substrate to the microtitre plate wells.
  Transfer the microtitre plate immediately to the plate reader set to read at an optical density of 405 nm and 37°C. Mix.
- **RATE 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.
- **END POINT ASSAY**
  Incubate with the kallikrein substrate for exactly 5 minutes, read optical densities, or add 25µl volumes of 50% acetic acid to stop the reaction and read the optical densities at 405 nm.

CALCULATION

Subtract 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 the corrected optical densities of the standards against the PKA standard values (Lin/Lin Standard Curve), or Log optical densities against Log PKA standard values (Log/Log Standard Curve). A polynomial regression plot is recommended for the calibration curve, using either an Endpoint or Rate assay.





Calculate the PKA values of the test samples from the Standard Curve or Log/Log Standard Curve and multiply the values obtained by 2.0 because of the dilution of the test sample with standard/sample diluent. As the Control plasma is tested un-diluted no correction is required.

Any test samples with PKA values greater than 32 IU/ml must be further diluted with standard/sample diluent and re-tested until an optical density value is obtained that falls within 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.

PERFORMANCE

STANDARDISATION

The assay kit is standardised against the 2nd International Standard for PKA [1,2].

QUALITY CONTROL

A mid level PKA control is provided with the kit to verify the validity of the calibration curve and sample results. The target range of PKA values for the kit control is included with the kit.
Additional, high and low level accuracy controls are also available as separate products, and it is recommended that these are included with each batch of tests.

REF PW51005 Just Positive™ Prekallikrein Activator (PKA) Control 5x0.5ml

REF PW52005 High Positive Prekallikrein Activator (PKA) Control 5x0.5ml

PRECISION

- **Inter-Assay**

Sample 1	5.8 IU/ml	8.8%
Sample 2	15.5 IU/ml	8.2%

- **Intra-Assay (n=20)**

Sample 1	5.8 IU/ml	6.3%
Sample 2	15.5 IU/ml	5.4%

RECOVERY

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.

SOURCES OF ERROR

- To obtain reliable, accurate and consistent results adhere strictly to the instructions in this insert.
- Store the kit at 4°C. Do not use past the expiry date.
- Use clean pipette tips for each reagent or specimen manipulation.
- Standard incubation times MUST be adhered to as any variation can cause variable results.

WARNINGS & PRECAUTIONS

- The PKA standard and control have been prepared from human sources and the sample/standard diluent contains material of animal origin, so both should be treated as potentially infective agents and handled accordingly.
- The buffer contains the preservative sodium azide, a poisonous compound. Do not pipette by mouth
- Care should be taken when handling any reagents contained within this kit.

LITERATURE

1. Longstaff C, Behr-Gross M-E, Daas A, Lackner F. An international collaborative study to replace the 1st international standard for prekallikrein activator. Vox Sanguinis 2005; 88:143-151.

2. Longstaff C, Behr-Gross M-E, Daas A, Lackner F. Collaborative Study to Establish a new Biological Reference Preparation for Prekallikrein Activator. Pharmeuropa-Bio, 2005-1, 1-11.

ALL REAGENTS AND MATERIALS ARE FOR IN VITRO USE ONLY.

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