

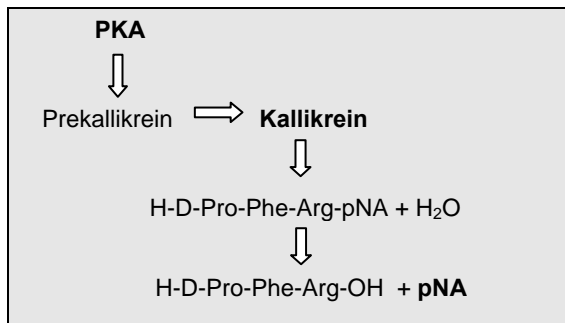
# PreKallikrein Activator Assay Kit

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 50 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 2-8°C before use.

- 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 -20°C for 6 months.
- 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 4°C, or at -20°C for 6 months. Stability after dilution: 6 hours at room temperature or 24 hours at 4°C
- PKA Standard 50 IU/ml (1x 1.0ml)**  
Reconstitute in 1.0 ml of sample/standard diluent. This gives a PKA concentration of 50IU/ml. Store at 4°C before use or for up to 8 hours or at -20°C for 6 months.
- Buffer A Concentrate (1x6ml)**  
Tris-HCl buffer (100 mmol/l Tris) containing NaCl (24 mmol/l). Store at 4°C.  
The vial contains 6ml of concentrated buffer. Before use dilute the contents of each vial with sterile

distilled water to give a final volume of 12ml for each vial. (Buffer A)

- Buffer B**  
Dilute 1 ml of Buffer A with 9 ml sterile distilled water.
- Sample/Standard Diluent**  
Dissolve vial contents in 6 ml sterile distilled water. Store at room temperature for up to 8 hours or for longer term storage at -20°C for 6 months.
- Microtitre Plates**  
Two clear plastic 96x8 well microtitre plates are supplied with the kit

## STANDARD CURVE

- Standard Curve**  
Dilute the PKA standard with standard/sample diluent to give PKA values of 0, 1.56, 3.125, 6.25, 12.5 and 25.0 IU/ml as follows:

PKA Concentration	PKA Standard	Standard/Sample diluent
IU/ml	µl	µl
0	0	200
1.56	12.5	387.5
3.125	25	375
6.25	50	350
12.5	100	300
25.0	100	100

## TEST SAMPLES

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

## ASSAY METHOD

- Step A for standards and test samples**  
Into 1.5 ml polypropylene Eppendorf tubes pipette: 25 µl volumes of each PKA standard dilution or diluted test samples.  
Add 50 µl PreKallikrein solution, mix and cap.
- Step A for standards and test sample blanks.**  
Into 1.5 ml polypropylene Eppendorf tubes pipette: 25 µl volumes of each PKA standard dilution or diluted test samples.  
Add 50 µl volumes of Buffer A, mix and cap.  
Incubate all tubes at 37°C for exactly 45 minutes

- **Step B for standards, test samples and test sample blanks**

Pre-warm diluted kallikrein substrate at 37°C

- Into microtitre plate wells in duplicate pipette: 25 µl volumes from all of the above incubates  
Using a multipipette add 100 µl diluted kallikrein substrate
- Transfer the microtitre plate immediately to a plate reader set to read at an optical density of 405 nm and 37°C.

#### RATE ASSAY

Measure the absorbance change for 2 to 3 minutes depending upon your laboratory instrumentation and protocols.

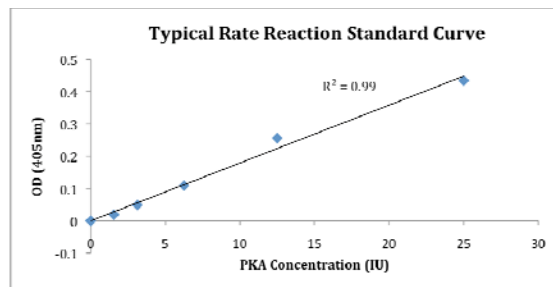
#### END POINT ASSAY

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 resulting corrected optical densities of the standards against PKA standard values (IU/ml). See typical standard curve below.



Calculate the PKA values of the test samples from the standard curve by multiplying the value obtained by 2 because of the dilution of the test sample with standard/sample diluent.

Any test samples with PKA values greater than 50 IU/ml (25 IU/ml in the assay) must be further diluted with standard/sample diluent and re-tested until an optical density value is obtained that falls on 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 2<sup>nd</sup> International Standard for PKA<sup>(1,2)</sup>. 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.

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

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

##### PRECISION

- Inter-Assay (manual technique)

Sample 1	4.4 IU/ml	8.6%
Sample 2	12.7 IU/ml	8.3%

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

Sample 1	4.4 IU/ml	6.5%
Sample 2	12.7 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 has 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 1<sup>st</sup> 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.**



**Pathway Diagnostics Ltd**  
Curtis Road, Dorking, RH4 1EJ, UK  
Tel: 0044 1306 888777  
[www.pathwaydiagnostics.com](http://www.pathwaydiagnostics.com)

#### Distribution:

##### TECOmedical AG

##### Headquarters TECOmedical Group

Gewerbestrasse 10

4450 Sissach

Phone +41 (0) 61 985 81 00

Fax +41 (0) 61 985 81 09

[info@tecomedical.com](mailto:info@tecomedical.com)

[www.tecomedical.com](http://www.tecomedical.com)

# PreKallikrein Activator Assay Kit

An assay kit for the determination of Prekallikrein Activator in biological fluids



PW301EP

For Research Use Only

## QUICK GUIDE – RATE METHOD

Reconstitute Prekallikrein in 2.5ml, Substrate in 1ml and Albumin in 6ml dist. water  
Reconstitute PKA standard in 1ml Albumin solution  
Dilute Buffer A concentrate in 6ml dist. water to give 12ml in total  
Dilute 1ml of Buffer A with 9ml dist. water to give Buffer B

Dilute 1ml **Substrate solution** with 9ml **Buffer B** before use

Prepare serial dilution of **PKA standard** in **Albumin** solution from 1.56 to 25 IU/ml

Dilute **100 µl** of test **samples** with **100 µl** of **Albumin solution**

Add **25 µl** of each **PKA standard dilution** or **diluted test sample** to **50 µl** **Prekallikrein solution**. Mix and cap

Prepare **Blanks** by adding **25 µl** of each **PKA standard dilution** or **diluted test sample** to **50 µl** **Buffer A**. Mix and cap

Add **25 µl** of each **standard, test sample** and their **blanks** in **duplicate** into microtitre plate wells

Add **100 µl** of diluted Substrate, pre-warmed to 37°C to each well

Incubate at 37°C and record the **ΔA/min** change for **2 to 3 minutes** at **405nm**

Correct the **ΔA/min** by **subtracting** the value for the corresponding **blank**  
Plot corrected OD's against PKA standard values (IU/ml)  
Calculate the test samples values from the curve and multiply by 2 to correct for the dilution in Albumin solution



Please read kit instruction before using this Quick Guide