

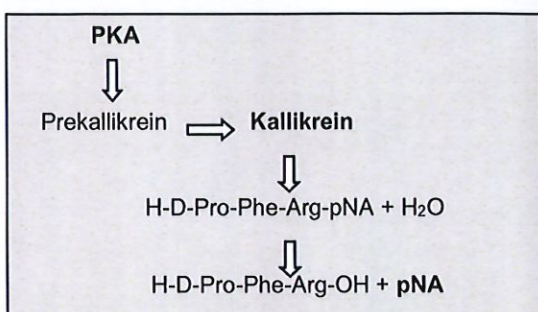
# PreKallikrein Activator Assay Kit

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 2-8°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. Mix well before use.
- 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 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 4°C before use for up to 8 hours, or freeze to -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 the vial with 6ml of sterile distilled water to give a final volume of 12ml in the vial. (Buffer A)

- Buffer B**  
Prepare Buffer B by diluting 1 ml of Buffer A with 9 ml sterile distilled water.
- 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 -20°C for 6 months.
- 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 4°C
- Microtitre Plates (x2)**  
The kit is supplied with two, clear plastic 96 well microtitre plates.

## STANDARD CURVE

- 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 IU/ml	PKA Standard µl	Standard/Sample diluent 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:

---

## Prekallikrein Activator (PKA) Positive Control

---

### Batch CC02202

The following control ranges were established using the Pathway Diagnostics PreKallikrein Activator Assay kits PW301EP and PW302EP, calibrated against the 2<sup>nd</sup> International standard for PKA:

Control	Batch	Mean IU/ml	Range IU/ml
Positive Control for PKA	CC02202	8.8	7.2 – 10.8