PreKallikrein Activator Assay Kit

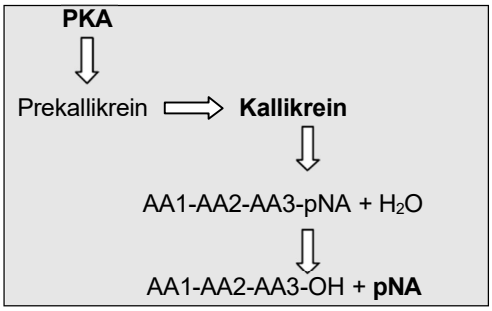
A chromogenic assay kit for the determination of Prekallikrein Activator in biological fluids

PRODUCT CODE: PW30100

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 25 IU/ml

Reagents

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 or at -20ºC for 6 months. Mix well before use.
2. **Kallikrein Substrate. PW-2301 (2x 5ml)**
   10µmol/vial plus mannitol. Reconstitute in 5 ml sterile distilled water and then **dilute 5 ml with 5 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 50 IU/ml (1x 1ml)**
   Reconstitute in 1.0 ml of standard/sample diluent. This gives a PKA concentration of 50IU/ml. Store at 4ºC before use or for up to 8 hours or at -20ºC for 6 months.
4. **Buffer A concentrate (1x 6ml)**
   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. **Standard/Sample diluent (2x 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. **Microtitre Plates (x2)**
   Two clear plastic 96 well microtitre plates are supplied with the kit

Preparation of Standard Curves

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 of 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 37ºC 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 to incubate @ 37°C.

END POINT ASSAY

Mix and then incubate for exactly 7 minutes. Read the optical densities or add 25µl volumes of 50% acetic acid to stop the reaction and then read the optical densities.

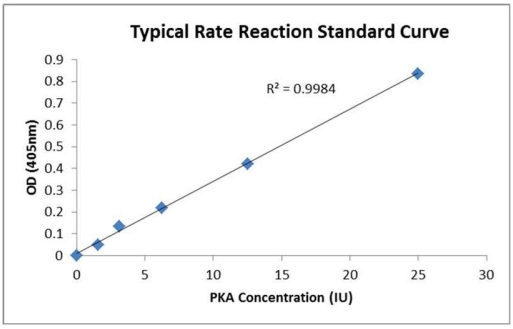
RATE ASSAY

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

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 curves in figures.



Calculate the PKA values of the test samples from the standard curve 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 25 IU/ml must be further diluted with standard/sample diluent and re-tested until an optical density value is obtained which 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 **Prekallikrein Activator (PKA) Assay kit** is standardised against the 2nd International Standard for PKA [1, 2]. It is recommended that the PKA positive level 1 and 2 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	5.8 IU/ml	8.1%
Sample 2	12.5 IU/ml	7.6%

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

Sample 1	5.8 IU/ml	6.3%
Sample 2	12.5 IU/ml	5.7%

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 albumin have been prepared from human sources and 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.

1. 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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