PATHWAY DIAGNOSTICS

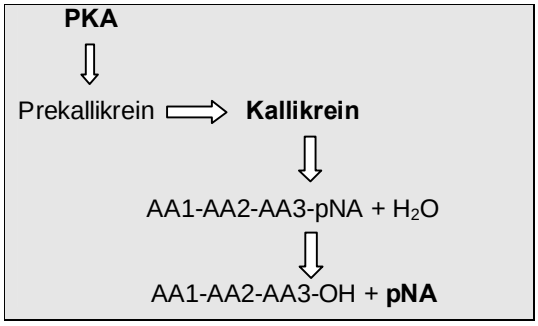
PreKallikrein Activator Assay IG Kit

A chromogenic assay kit for the determination of Prekallikrein Activator (PKA) in Human Immunoglobulin Preparations.

PRODUCT CODE: PW30200 50 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.

Conventional PKA assays of immunoglobulin fractions are often susceptible to high test blank levels, with blanks having higher values than those of the test sample. To eliminate this problem this kit includes an additional blanking step.

KIT CONTENTS

The kit should be stored at 2-8°C 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 -20°C 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 4°C, or at -20°C for 6 months. Stability **after dilution**: 6 hours at room temperature or 24 hours at 4°C
3. **PKA Standard 50 IU/ml (1x 1ml)**
   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.
4. **Buffer A Concentrate (2x 6ml)**
   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)
5. **Buffer B**
   Dilute 1 ml of Buffer A with 9 ml sterile distilled water.
6. **Sample/Standard 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 -20°C for 6 months.
7. **Immunoglobulin Pre-treatment Reagent. (1x 5ml)**
   All immunoglobulin fractions must be pre-treated with this reagent before being tested.
   The reagent is ready to use. Store at 4°C
   Pre-treatment schedule:
   Add 100 µl of pre-treatment reagent to 900 µl of immunoglobulin sample in a plastic tube. Mix well and assay as per the test schedule shown in the kit
8. **Blank Activity Blocking Reagent (BABR) (1x 1ml)**
   Reconstitute in 1.0 ml sterile distilled water (Stock Solution). To this solution add 11ml of diluted Buffer A (1 part buffer A plus 1 part sterile distilled water). Mix and use within 4 hours at room temperature or store in 3.0 ml aliquots at -20°C.
9. **Microtitre Plates. (x2)**
   Two clear plastic 96 well microtitre plates are supplied with the kit

STANDARD CURVE

1. **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 pre-treated immunoglobulin 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 normal 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 A for additional test sample blanks. (Not required for standards)**
  Into 1.5 ml polypropylene Eppendorf tubes pipette:

  25 µl volumes of diluted test samples.

  Add 50 µl volumes of Blank Activity Blocking Reagent (BABR), mix and cap.

  Incubate all tubes at 37°C for exactly 45 minutes
- **Step B for standards, test samples, test sample blanks and BABR 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.

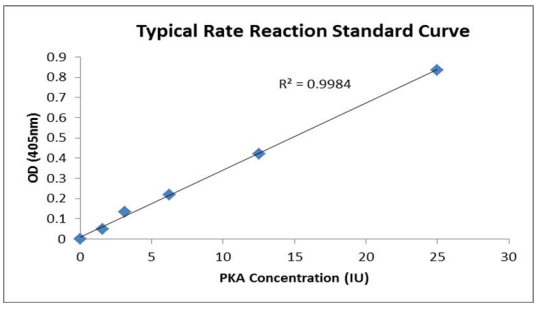
RATE ASSAY
Measure the absorbance change for 2 to 3 minutes depending upon your laboratory instrumentation and protocols.

END POINT ASSAY
Incubate with the kallikrein substrate for exactly 7 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 2.2 because of the pre-dilution during treatment and 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.

Subtract the optical densities obtained with the BABR from the optical densities obtained for the normal (Buffer A) blanks and the resulting value is the optical density due to any spontaneous activity in the immunoglobulin fractions. This value has to be subtracted from the optical density values obtained for the immunoglobulin test values (immunoglobulin incubated with prekallikrein). It is then possible to compare test results obtained with the EP method with those obtained after blocking blank activity.

PERFORMANCE

STANDARDISATION
The assay kit is standardised against the 2nd International Standard for PKA [1,2]. It is recommended that the PKA high and low positive accuracy controls designed for use with the **Prekallikrein Activator (PKA) Assay IG 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

- Recovery from human immunoglobulin preparations spiked with known PKA concentrations, (1 to 20 IU/ml), yielded on average 102% (87-113%) 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 A and the Immunoglobulin pre-treatment reagent contain 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.

Pathway Diagnostics Ltd
Curtis Road, Dorking, RH4 1EJ, UK
Tel: 0044 (0)1306 888777
www.pathwaydiagnostics.com