New Test Procedure
Ths kit is calibrated against the 3rd IS for PKA, and the test method is suitable for automates.

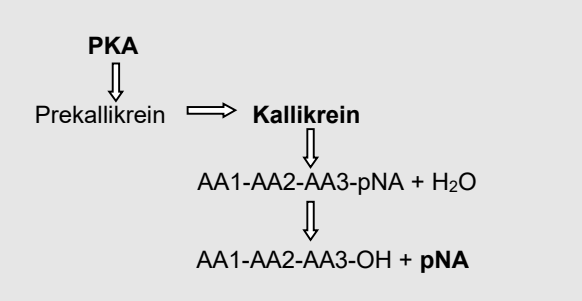
PreKallikrein Activator Assay Ig Kit

An assay kit for the determination of Prekallikrein Activator (PKA) in Human Immunoglobulin Preparations

Product Code: PW30200 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.

In conventional PKA assays of immunoglobulin fractions, high-test blanks can sometimes occur, with values higher than those of the test samples. To eliminate this problem this kit includes an additional blanking step.

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 (18 – 24°C) before use for up to 24 hours. For longer term storage at -20ºC for 6 months. Mix well before use.
2. **Kallikrein Substrate PW-2301 (2x 10ml)**
   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**: 8 hours at room temperature, 24 hours at 4-8 ºC and 6 months at -20 ºC or below. Mix well before use.
3. **PKA Standard 50 IU/ml (1x 1ml)**
   Reconstitute in 1.0 ml of sample/standard diluent, leave for 5 min at room temperature and mix well. This gives a PKA concentration of 50 IU/ml. Store this at 4ºC before use for up to 8 hours, or freeze at -20ºC for 6 months.
4. **Buffer A Concentrate (2x 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 each vial with 6ml of 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 -20ºC 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 4ºC
   Pre-treatment schedule:
   Add 50 µl of pre-treatment reagent to 450 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. 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**
   Prepare a serial dilution of the 50 IU/ml PKA standard with standard/sample diluent to give PKA values of 3.125, 6.25, 12.5 and 25.0 IU/ml as follows:

PKA Concentration	PKA Standard	Standard/Sample diluent
IU/ml	µl	µl
3.125	25	375
6.25	50	350
12.5	100	300
25	100	100

TEST SAMPLES

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

ASSAY PROCEDURE

- **Step A: Testing standards, control 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: Blanks for standards, control 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 volumes of Buffer A

Transfer the microtitre plate immediately to a plate reader set at 37ºC. Mix, and incubate for exactly 10 minutes

- **Step B: Testing standards, test samples and 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 8 minutes, read optical densities, or add 25µl volumes of 50% acetic acid to stop the reaction and read the optical densities at 405 nm.
- **Step C: Repeat testing High OD Sample Blanks with Blank Activity Blocking Reagent (BABR)**
  If any immunoglobulin test samples give a high blank value, (as a guide: an O.D. greater than the 3.125IU Standard), an additional BABR blank test can be performed to correct for non-specific hydrolysis of the substate. There is no need to perform this step for the standards or kit control.
  - Into microtitre plate wells in duplicate pipette:
  - 25 µl volumes of diluted test samples.
  - Add 50 µl volumes of BABR, mix and cap.

  Transfer the microtitre plate immediately to a plate reader set at 37°C. Mix, and incubate for exactly 10 minutes

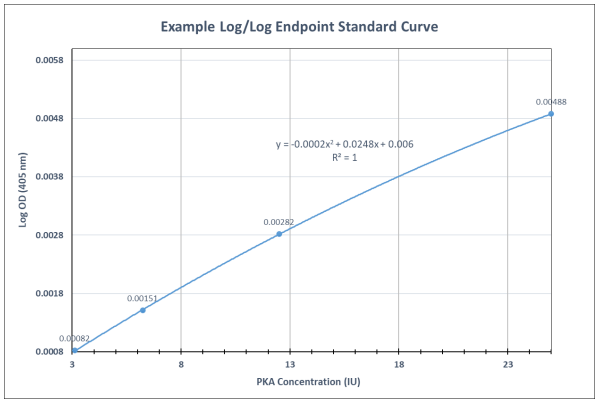
  Repeat Step B

CALCULATION

Subtract the optical densities (OD) obtained for the blanks of the standards, control and test samples from the OD values obtained for the standards, control and test samples.

If the O.D values for the test blank samples are high, a BABR blank test should also be performed on these samples. The BABR blank should then be subtracted from the O.D. values of the test sample blanks. The resulting corrected O.D. values of the high OD test sample blanks should then be subtracted from the O.D. values of the 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). An example of a rate assay Log/Log calibration curve is shown below.



Calculate the PKA values of the test samples from the Standard Curve or Log/Log Standard Curve and multiply the values obtained by 2.2 to allow for the dilution of the test sample with standard/sample diluent.

Any test samples with PKA values greater than 50 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 3rd International Standard for PKA [1,2,3].

QUALITY CONTROL

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	8.1%
Sample 2	12.5 IU/ml	7.6%

- **Intra-Assay (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 beyond 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.
- Care should be taken when handling any reagents contained within this kit.

LITERATURE

1. WHO/BS/2019.2357. WHO 3rd IS for Prekallikrein Activator
2. Fox B, Regourd E, Rigsby P, Longstaff C, Terao E. Joint Collaborative study for the establishment of the WHO 3rd International Standard for PKA in albumin. Pharmeur Bio & Sci Notes 2020. In preparation. WHO/BS/2019.2357.
3. Longstaff C, Behr-Gross M-E, Daas A, Lackner F. Collaborative Study to Establish a new Biological Reference Preparation for PKA. Pharmeuropa-Bio, 2005-1, 1-11.

ALL REAGENTS AND MATERIALS ARE FOR IN VITRO USE ONLY.

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