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

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)

5. Buffer B
Prepare Buffer B by diluting 1 ml of Buffer A with 9 ml sterile distilled water.

6. 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.

7. 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

8. Microtitre Plates (x2)
The kit is supplied with two, clear plastic 96 well microtitre plates.

STANDARD CURVE

1. 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:

<table>
<thead>
<tr>
<th>PKA Concentration</th>
<th>PKA Standard</th>
<th>Standard/Sample diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>IU/ml</td>
<td>µl</td>
<td>µl</td>
</tr>
<tr>
<td>2.0</td>
<td>25</td>
<td>375</td>
</tr>
<tr>
<td>4.0</td>
<td>50</td>
<td>350</td>
</tr>
<tr>
<td>8.0</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>16.0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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:
• 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 37°C. Mix, and incubate for exactly 10 minutes
• Step B for standards, test samples and test 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 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 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). A polynomial regression plot is recommended for the calibration curve, using either an Endpoint or Rate assay.

PERFORMANCE
STANDARDISATION
The assay kit is standardised against the 2nd International Standard for PKA [1,2].

QUALITY CONTROL
A mid level PKA control is provided with the kit to verify the validity of the calibration curve and sample results. The target range of PKA values for the kit control is included with the kit. Additional, 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  8.8%
  Sample 2 15.5 IU/ml 8.2%
• Intra-Assay (n=20)
  Sample 1  5.8 IU/ml  6.3%
  Sample 2 15.5 IU/ml 5.4%

RECOVERY
The recovery from Human Albumin solutions spiked with known PKA concentrations (5 to 291IU/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 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.
• 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

All reagents and materials are for in vitro use only.

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