UNITEST™ PKK

Amidolytic Substrate Assay for Prekallikrein

FOR RESEARCH USE ONLY

This kit is designed for research use only, for the measurement of plasma prekallikrein (PKK) in human plasma. Prekallikrein activator reagent (CONT-ACT PK™ converts prekallikrein to plasma kallikrein, which is able to cleave a specific tri-peptide chromogenic substrate and liberate p-nitroaniline (pNA), which can be measured photometrically. The pNA concentration is directly proportional to the plasma kallikrein concentration[1].

REAGENTS

Store the unopened kit at 4°C until reconstituted. It is stable until the stated expiry date.

1. *Unitrate™PKK, Plasma Kallikrein Substrate*
10µmol/vial MBz-Pro-Phe-Arg-pNA, plus mannitol. Dissolve in 5ml sterile distilled water, transfer to a suitable plastic tube or bottle and dilute with a further 5ml sterile distilled water. Stable for at least 6 months at 4°C if kept free from contamination. It may also be stored in aliquots at below -20°C.

2. *CONT-ACT PK™*
CONT-ACT PK™ is a lyophilised preparation containing a mixture of ellagic acid, phospholipid, a plasma fraction containing factor XII and high molecular weight kininogen, plus buffer salts and stabilisers. Dissolve in 5ml distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

3. *Buffer Concentrate*
Dilute the buffer concentrate with distilled water in the ratio of 1:9, to provide a sufficient volume of buffer for the tests required. This gives a buffer of 0.05M Tris-HCl, pH 8.0, store at 4°C. Diluted buffer should be used within 24 hours.

4. *Standard Plasma*
Add 1ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable for 8 hours at 4°C.

Required but not provided: acetic acid 50%, 10ml plastic tube for substrate dilution.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9ml) is mixed with 0.106M tri-sodium citrate (1ml) and centrifuged at 2000 x g for 15 minutes at room temperature. The plasma samples should be removed with plastic pipettes within two hours of blood collection and should be assayed immediately or stored frozen at -20°C.

PREPARATION OF THE STANDARD CURVE

The standard plasma is diluted with buffer as follows:

Standard %	Plasma	Buffer
150	75µl	2500µl
100	50µl	2500µl
From the 100% standard prepare:		
75	600µl	200µl
50	400µl	400µl
25	200µl	600µl
0	Use buffer alone	

Dilute 50µl of each test plasma with 2500µl buffer.

ASSAY METHOD

Warm the substrate and activator to 37°C and keep the plasma dilutions at room temperature. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer	200µl

Incubate at 37°C for 2 minutes, add:

CONT-ACT PK™	200µl

Mix and incubate at 37°C for 2 minutes, add:

Unitrate™ PKK, Kallikrein Substrate	200µl

Mix and record the change in optical density per minute at 405nm (rate assay), or incubate for exactly 30 minutes at 37°C, add:

Acetic acid (50%)	200µl

Mix and read optical density at 405nm (end point assay).

MICROTITRE METHOD

Follow the manual method above, but pipette 50µl volumes of each plasma dilution and reagent into the wells of a 96 well polystyrene microtitre plate. Care must be taken to ensure adequate mixing after each reagent addition.

ACL-3000 METHOD

Place analyser cups containing the dilutions of standard and test samples in the autosampler tray, so that the standard dilutions occupy positions 1-7, and the test samples positions 8-18.
Place a sample cup of assay buffer in the position marked "DIL".
Place CONT-ACT PK™ (volume depending on the size of the assay run) in reagent position 2.
Place Unitrate™ PKK in reagent position 3.

From the *Research Mode*, select *Chromogenics* and programme the assay conditions into the "Loading Conditions" and "Incubation and Acquisition Conditions" as follows:-

Sample Volume	50µl
Reagent Volume Position 2	50µl
Reagent Volume Position 3	50µl
Reaction Time	300s
Inter Ramp Interval	3s
Delay Time	30s
Acquisition Time	300s
Speed	1200 rpm

Start the analysis after making the routine instrument checks, and prepare the microcomputer to receive the data.

CALCULATION
With the end point assay, if the test plasmas have high bilirubin levels, are lipaemic or have visible haemolysis, blanks must be performed. For the blanks take 200µl volumes of diluted plasma, add 400µl buffer and 200µl acetic acid, and mix (for the microtitre method, reduce these volumes by a factor of four). The A_{405} values for the blanks are subtracted from the test values before reading the prekallikrein values from the standard curve.
Plot the results as A_{405} against percentage prekallikrein for the standard plasma dilutions and the values for the test plasma from the standard curve. The values can be expressed either as a percentage or in units per ml (U/ml) by applying the formula:

$$\text{PKK (U/ml)} = \frac{\text{\% Activity x Potency of Standard}^{*}}{100}$$

*The potency value of the standard plasma for plasma prekallikrein (lot UD-0137-1090) is 1.08 U/ml.

PERFORMANCE CHARACTERISTICS
The standard curve is linear up to 150%. The intra-assay coefficient of variation = 4% at 1.00 U/ml. The detection limit = 0.05 U/ml.

INTERPRETATION
Normal Range 0.67 - 1.36 U/ml

HAZARD WARNING
All materials of human origin were tested and found negative for the presence of HBsAg, anti-HB core, HCV antibodies and anti-HIV antibody. However, as with all preparations of human origin, these products cannot be assumed to be free from infectious agents and suitable precautions should be taken in their use and disposal.

NOTE
The recommended standard and test sample dilutions may vary between different batches of this kit, owing to differences in the specific activity of some batches of reagents.

REFERENCES
1. Gallimore MJ & Friberger P. Thromb Res 1982; 25: 293-298.

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