

# Unitest™ KKA

## Chromogenic Substrate Assay For Kallikrein-Like Activity In Human Plasma

This kit is designed for research purposes only, for the determination of kallikrein-like activity in human plasma<sup>1,2</sup>. This activity is predominantly due to kallikrein bound to alpha-2-macroglobulin. Plasma is diluted with buffer and kallikrein-like activity is measured using a chromogenic peptide substrate for plasma kallikrein. Cleavage of the substrate liberates p-nitroaniline (pNA), which can be measured photometrically. Kallikrein-like activity can be calculated from the amount of pNA released.

### REAGENTS

The reagents should be stored at 4°C until reconstituted.

#### 1. Unitest™ 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 below -20°C.

#### 2. 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, 0.1M NaCl, pH 8.0, store at 4°C. Diluted buffer should be used within 24 hours.

#### 3. High Activity Standard Plasma

Add 0.5ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable at room temperature for 4 hours, do not refrigerate.

#### 4. Low Activity Standard Plasma

Add 1ml distilled water, leave for 5 minutes at room temperature and then mix gently until completely dissolved. Stable at room temperature for 4 hours, do not refrigerate.

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

### BLOOD COLLECTION AND PREPARATION OF PLASMA

Blood (9ml) is mixed with 0.106M tri-sodium citrate (1ml) and centrifuged at 2000g 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.

**NOTE:** With plasma samples containing heparin, the heparin must be neutralised with protamine sulphate or protamine chloride before freezing (1mg protamine neutralises approximately 100 IU heparin).

### STANDARD AND TEST DILUTIONS

Dilute 100µl of High Activity Standard Plasma, Low Activity Standard Plasma and test plasmas with 1000µl buffer in plastic tubes at room temperature.

### ASSAY METHOD

Warm the substrate to 37°C and keep the plasma dilutions at room temperature.

Into plastic tubes pipette:

Diluted plasma 400µl

Incubate at 37°C for 3 minutes, add:

Plasma Kallikrein Substrate 200µl

Mix and incubate at 37°C for 20 minutes, add:

Acetic acid (50%) 200µl

Mix immediately.

Prepare plasma blanks by adding the reagents in reverse order without incubation, substituting buffer for substrate. Read the absorbance of the test samples and blanks in a spectrophotometer at 405nm. Subtract the absorbance values for the blanks from the test values.

### MICROTITRE METHOD

Follow the manual method above, but reduce the volumes of each plasma dilution to 100µl and each reagent to 50µl, and pipette them into the wells of a 96 well polystyrene microtitre plate. Care must be taken to ensure adequate mixing after each reagent addition.

### CALCULATION

Multiply the optical density values by 164; this gives kallikrein-like activities in U/l. For microplate assays, use a multiplication factor of 282 to obtain U/l.

## **INTERPRETATION**

The Low Activity Standard Plasma gives an activity similar to a plasma where low kallikrein-like activities are present. The High Activity Standard Plasma gives high kallikrein-like activity. Plasma samples from normal subjects should give a value close to that for the Low Activity Standard Plasma. Plasma samples from patients in whom plasma kallikrein has been activated will give values higher than the value for the Low Activity Standard Plasma. Elevated kallikrein-like activities are seen in cardiopulmonary bypass and septic shock or other conditions where activation of the kallikrein system occurs.

## **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. Simple chromogenic peptide substrate assays for determining prekallikrein, kallikrein inhibition and kallikrein 'like' activity in human plasma. *Thromb Res* 1982; 25: 293-298.
2. Friberger P. Chromogenic peptide substrates. *Scand J Clin Lab Invest* 1982; 42 (Suppl. 162): 40.

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**UNICORN DIAGNOSTICS Ltd,**  
Registered Office: Sandettie House, Hawkesdown Estate,  
Walmer, Kent CT14 7PH.

Tel / FAX +44 (0)20 8530 4181

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