

CHROMOGENIC SUBSTRATE ASSAY FOR INHIBITORS OF PLASMA KALLIKREIN IN HUMAN PLASMA

This kit is designed for the determination of inhibitors of plasma kallikrein in human plasma. Plasma kallikrein is added to diluted plasma and during an incubation period it complexes with plasma inhibitors. The residual plasma kallikrein activity is measured by its ability to cleave a chromogenic peptide substrate and liberate p-nitroaniline (pNA). The concentration of pNA is measured photometrically, and is inversely proportional to the plasma inhibition of kallikrein¹.

REAGENTS

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

1. Human Plasma Kallikrein

Dissolve in 10ml sterile distilled water.

2. Kallikrein Substrate

Dissolve in 10ml sterile distilled water. Stable for 8 hours at 4°C and 6 months at -20°C, if free from contamination.

3. Buffer Concentrate

Dilute contents of vial with 90ml sterile distilled water, store at 4°C.

4. Standard Plasma

Dissolve in 1ml distilled water. Stable for 8 hours at 4°C.

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.

PREPARATION OF THE STANDARD CURVE

Dilute the standard plasma with buffer as follows:

STANDARD %	PLASMA	BUFFER
150	150µl	850µl
100	100µl	900µl
From the 100% standard prepare:		
75	300µl	100µl
50	200µl	200µl
25	100µl	300µl
0	Use buffer alone	

Dilute 100µl of each test plasma with 900µl buffer.

ASSAY METHOD

Have the substrate at 37°C. Into siliconised semi-micro cuvettes, siliconised glass or plastic tubes pipette:

Plasma dilution or buffer 200µl

Incubate at 37°C for 2 minutes, add:

Plasma Kallikrein 200µl

Mix and incubate at 37°C for 5 minutes, add:

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).

CALCULATION

For the end point assay, prepare blanks by substituting 400µl buffer for the plasma kallikrein and kallikrein substrate. Subtract the blank values from the test values. Plot the results as log A_{405} against percentage kallikrein inhibition for the standard plasma dilutions and read the values for the test plasmas from the standard curve. The final

results can be expressed either as a percentage of standard, or in units per ml (U/ml) by applying the formula:

Plasma Kallikrein Inhibitors (U/ml) =

$$\frac{\% \text{ Activity} \times \text{Potency of Standard}}{100}$$

The potency value of the standard plasma for plasma kallikrein inhibitors (lot UD-0137-0260) is 1.00 U/ml.

PERFORMANCE CHARACTERISTICS

The assay is linear up to 150%, with a sensitivity limit of 5%. The intra-assay coefficient of variation is 5% at 1.00 U/ml.

INTERPRETATION

Normal Range 0.85 - 1.29 U/ml (85-129%)

The major plasma inhibitor of kallikrein is C1-inhibitor², although AT-III³, α_2 -macroglobulin⁴ and protein C inhibitor⁵ (PCI-1) contribute, and an uncharacterised low molecular weight inhibitor has been reported⁶.

HAZARD WARNING

All materials of human origin were tested and found negative for the presence of HBsAG 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.

REFERENCES

1. Gallimore MJ. Chromogenic peptide substrate assays for determining components of the plasma kallikrein system. *Scand J Clin Lab Invest* 1985; 45: 127-132.
2. van der Graff F, Koedman JA, Bouma BN. Inactivation of kallikrein in human plasma. *J Clin Invest* 1983; 71: 149-158.

3. Lahiri B, Bagdasarian A, Mitchell B, et al. Antithrombin-heparin cofactor: an inhibitor of human plasma kallikrein. *Arch Biochem Biophys* 1976; 175: 737-744.

4. Harpel PC. Human plasma alpha 2-macroglobulin. An inhibitor of plasma kallikrein. *J Exp Med* 1970; 132: 329-352.

5. Meijers JCM, Kanters DHA, Vlooswijk RAA, van Erp HE, Hessing M, Bouma BN. Inactivation of human plasma kallikrein and factor XIa by protein C inhibitor. *Biochemistry* 1988; 27: 4231-4237.

6. Gallimore MJ, Amundsen E, Larsbraaten M, Lyngaas K, Faried E. Studies on plasma inhibitors of plasma kallikrein using chromogenic peptide substrate assays. *Thromb Res* 1979; 16: 695-703.

CATALOGUE NUMBER: 0073

PRODUCT: Plasma Kallikrein Inhibitors Kit
UNICORN DIAGNOSTICS Ltd,
London, UK
Tel (+44) 181 559 1006

17/06/93