

Unitest™ HEPCO II

Amidolytic Substrate Assay for Heparin Cofactor II

This kit is designed for the determination of heparin cofactor II (dermatan sulphate cofactor¹) in human plasma. Dermatan sulphate is added to dilute plasma to facilitate the inhibition of purified human thrombin by heparin cofactor II. The residual thrombin activity is then measured using a chromogenic peptide substrate for thrombin. The concentration of pNA cleaved from the substrate is measured photometrically and is inversely proportional to the heparin cofactor II concentration.

REAGENTS

The kit reagents should be stored at 4°C until reconstituted; they are stable until the stated expiry date.

1. Human Thrombin

Each vial contains approximately 7 IU human thrombin (by amidolytic assay), plus stabilisers. Dissolve in 5ml distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

2. Dermatan Sulphate

Each vial contains approximately 1.5mg dermatan sulphate. Dissolve in 5ml of distilled water. Stable for 8 hours at 4°C and 6 months at -20°C.

3. Unitrate™TH - Thrombin Substrate

10µmol/vial 2AcOH.H-D-CHG-Gly-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 -20°C.

4. 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, 0.15M NaCl, 7.5mM Na₂EDTA, pH 8.2, containing 2mg/L polybrene. Store at 4°C. Diluted buffer should be used within 24 hours.

5. Standard Plasma

Add 1.0ml 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: 50% acetic acid, 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 must be removed with plastic pipettes within two hours of blood collection and should be assayed immediately or frozen at -20°C.

PREPARATION OF THE STANDARD CURVE

The standard plasma is diluted with buffer as follows:

Standard %	Plasma	Buffer
150	45µl	3000µl
100	30µl	3000µl
From the 100% standard prepare:		
75	600µl	200µl
50	400µl	400µl
25	200µl	600µl
0	Use buffer alone	

Dilute 30µl of each test plasma with 3000µl buffer.

ASSAY METHOD

Have the substrate at 37°C. Into plastic tubes, siliconised glass tubes or siliconised microcuvettes, pipette:

Buffer or plasma dilutions 100µl

Dermatan Sulphate 100µl

Mix and incubate at 37°C for 5 minutes,

Add Human Thrombin 100µl

Mix and incubate at 37°C for 5 minutes,

Add Thrombin Substrate 200µl

Mix and record the change in optical density at 405nm (Rate assay), or Incubate for exactly 5 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 40µl volumes of plasma dilution, dermatan sulphate and human thrombin and 80µl volumes of substrate and acetic acid into the wells of a 96 well polystyrene microtitre plate, following the same incubation times. Care must be taken to ensure adequate mixing after each reagent addition.

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 100µl volumes of diluted plasma, add 400µl buffer and 200µl acetic acid, and mix (for the microtitre method, use 40µl diluted plasma, 160µl buffer and 80µl acetic acid). The A₄₀₅ values for the blanks are subtracted from the test values before reading the heparin cofactor II values from the standard curve.

Plot the results as A₄₀₅ against percentage heparin cofactor II for the standard plasma dilutions and read the values for the test plasmas from the standard curve. The values can be expressed either as a percentage or in units per ml (U/ml) by applying the formula:

$$\text{Heparin Cofactor II (U/ml)} = \frac{\% \text{ Activity} \times \text{Potency of Standard}^*}{100}$$

*The potency of the standard plasma for HCII (lot 0137-0800) is 1.12 U/ml.

PERFORMANCE CHARACTERISTICS

The assay is linear up to 150%, with a sensitivity limit of 10% (0.10 U/ml). The intra-assay coefficient of variation is 4% at 1.00 U/ml.

INTERPRETATION

Normal Range 0.65 - 1.35 U/ml.

Increased levels may be detected in women receiving oral contraceptives, and in acute phase reactions²⁻⁴. Decreased levels may occur in multi-systems organ failure, DIC, and after major surgery⁵. Deficiency of heparin cofactor II has been associated with recurrent thrombosis⁶⁻⁹.

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.

REFERENCES

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5. Tollefsen DM. In: Heparin - Chemical and Biological Properties, Clinical Applications, Lane DA & Lindahl U (editors), Edward Arnold, London, 1989, 257-275.
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7. Sie P et al. Lancet 1985; ii: 414-416.
8. Simioni P et al. Blood Coagulation and Fibrinolysis 1990; 1: 351-356.
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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.

PRODUCT NUMBER: 0036

PRODUCT: Unitest™ HepCo II

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