

Unitest™ FXIIa

Amidolytic Substrate Assay For Factor XIIa-like Activity In Human Plasma

This kit is designed for research use only, for the measurement of factor XIIa (FXIIa)-like activity in human plasma. FXIIa-like activity is predominantly the activity of α FXIIa bound to α_2 -macroglobulin. Plasma is diluted with buffer and FXIIa-like activity is measured using a chromogenic peptide substrate for α FXIIa. Cleavage of the substrate liberates p-nitroaniline (pNA), which can be measured photometrically. FXIIa-like activity can be calculated from the amount of pNA released.

REAGENTS

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

1. Unitest™ FXII, factor XIIa substrate

10 μ mol/vial 2AcOH.H-D-CHT-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.

2. Kallikrein Inhibitor

Approximately 20mg soybean trypsin inhibitor, 0.18g mannitol and buffer salts. Dissolve in 10ml 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, 0.15M NaCl, pH 7.9. Mix 1ml of Kallikrein Inhibitor with 49ml diluted buffer to obtain a working solution. Store at 4°C; diluted buffer should be used within 24 hours.

4. 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.

5. Low Activity Standard Plasma

Add 1.0ml 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: 50% acetic acid, 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 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 100iu heparin).

STANDARD AND TEST DILUTIONS

Dilute 100 μ l of High Activity Standard Plasma, Low Activity Standard Plasma and test plasmas with 1000 μ l buffer containing Kallikrein Inhibitor, 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 400 μ l

Incubate at 37°C for 3 minutes, add:

FXIIa Substrate 200 μ l

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

Acetic acid (50%) 200 μ l

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 147, this gives FXIIa-like activities in u/l. For microplate assays, use a multiplication factor of 253 to obtain u/l.

INTERPRETATION

The Low Activity Standard Plasma gives an activity similar to a plasma where low FXIIa-like activities are present. The High Activity Standard Plasma gives high FXIIa-like activity. Plasma samples from normals should lie near to the value for the Low Activity Standard Plasma. Plasma samples where FXII has been activated will give values higher than the value for the Low Activity Standard Plasma.

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.

PRODUCT NUMBER: 0088

PRODUCT: Unitest™ Factor XIIa Kit

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THIS PRODUCT IS FOR RESEARCH USE ONLY

NOT FOR DIAGNOSTIC USE

NOTE:

Because of the low levels of FXIIa-like activity in normal plasma there is not a lot of colour development in this assay.

When using the kit in the microtitre format it is suggested that to obtain higher optical density readings use a 60 minute incubation time with the substrate. With this incubation time the factor for multiplication is 87.7.