CoaChrom® Aprotinin

Chromogenic Assay for Aprotinin

Ref.No. COA0135

For Research Use Only
Not For Use in Diagnostic Procedures
For *in vitro* Use Only

The kit is designed for the determination of Aprotinin in plasma. Plasma is treated with acetone to remove the effect of serine protease inhibitors and then diluted in buffer containing inhibitors of α_2-Macroglobulin and Factor XIIa. Purified plasma Kallikrein is added to acetone treated plasma or aqueous solutions containing Aprotinin, and after an incubation period it complexes with the Aprotinin in the test sample. Residual plasma Kallikrein activity is then 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 Aprotinin concentration.

REAGENTS

The unopened reagents should be stored at 2-8°C until reconstituted.

1. Chromogenic Kallikrein Substrate, 10 mL 1 vial
Dissolve with 10 mL distilled water. Stable for at least 6 months at 2-8°C.

2. Human Plasma Kallikrein, 10 mL 1 vial
Contains approximately 0.1 PEU (25-50 µg) of plasma kallikrein and stabilisers. Reconstitute with 10 mL distilled water. Stable for 8 hours at 2-8°C or 6 months if stored in aliquots at <-60°C.

3. Buffer Concentrate, 10 mL 2 vials
Dilute the buffer concentrate 1+9 with distilled water. This gives an assay buffer of 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.8. Store at 2-8°C. Diluted buffer should be used within 24 hours.

4. Aprotinin Standard, 1 mL 1 vial
Reconstitute with 1.0 mL distilled water. This gives a stock solution of about 2500 KIU/mL. Stable for 8 hours at 2-8°C or 6 months at -20°C.

5. Corn Trypsin Inhibitor (CTI), 10 mL 2 vials
Reconstitute with 10 mL distilled water. Stable for 8 hours at 2-8°C or 6 months at -20°C.

5a. Corn Trypsin Inhibitor + Buffer (CTI+Buffer)
Add 10 mL Corn Trypsin Inhibitor (Reagent 5) to 90 mL diluted buffer (3). Stable for 24 hours at 2-8°C.

6. Normal Plasma, 3 mL 1 vial
Reconstitute with 3.0 mL distilled water, leave for 5 minutes at room temperature and then gently mix until completely dissolved. Keep at room temperature and use within 4 hours or store at -20°C for up to 6 months.

Reagents required, but not provided: 20% acetic acid or 2% citric acid, acetone.

BLOOD COLLECTION AND PLASMA PREPARATION

Blood (9 mL) is mixed with 0.106 M Tri-sodium citrate (1 mL) 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 STANDARD CURVES

Separate standard curves are prepared for plasma samples and aqueous solutions.

Standard Curve for Plasma Samples

1. Primary Dilutions

From the Aprotinin Standard stock solution (Reagent 4) prepare the following dilutions:

	Aprotinin (KIU/mL)	Aprotinin Std. Stock (µL)	CTI+Buffer (µL)
A.	0	0	1000
B.	250	100	900
C.	500	100	400
D.	1000	100	150
E.	2500	use stock solution alone	

2. Secondary Dilutions:

Aprotinin (KIU/mL)	Aprotinin from 1. Primary Dilutions	Normal Plasma (µL)
0	50 µL A.	450
25	50 µL B.	450
50	50 µL C.	450
100	50 µL D.	450
250	50 µL E.	450

ACETONE TREATMENT

Mix 300 µL of each test plasma and Aprotinin Standard dilution with 100 µL acetone in a

polypropylene or siliconised glass test tube. Leave for 15 minutes at 4°C, and centrifuge at 2000g for 5 minutes, then dilute 300 µL acetone treated plasma with 1700 µL CTI+Buffer (5a) and keep on ice until assayed (within 1 hour).

Standard Curve for Aqueous Solutions of Aprotinin
Prepare primary dilutions A, B, C, D, E as above. Prepare secondary dilutions as for plasma, but substituting buffer containing Corn Trypsin Inhibitor (5a) for normal plasma. Dilute the aqueous Aprotinin test solutions appropriately, to bring the results into the range of the standard curve. No acetone treatment is required.

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

Plasma dilution or buffer	200 µL
Incubate at 37°C for 1 min, add:	
Human Plasma Kallikrein (2)	200 µL
Mix and incubate at 37°C for 5 min, add:	
Chromogenic Kallikrein Substrate (1)	200 µL
Mix and record the change in optical density per minute at 405nm (rate assay), or incubate for exactly 20 min at 37°C, add:	
Acetic acid or citric acid	200 µL

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

Microplate Method
Follow the manual method above, but pipette 50 µL volumes of each plasma dilution and reagent into the wells of a polystyrene microplate. Care must be taken to ensure adequate mixing after each reagent addition

Assay Blanks
Blanks should be prepared when Aprotinin is measured in plasma samples (to correct for differences in plasma colour and endogenous protease activities in plasma), by substituting 200 µL (50 µL for the microtitre method) of CTI+Buffer (Reagent 5a) for the Plasma Kallikrein.

CALCULATION
Subtract the blank A_{405} values from the standard (including the zero KIU/mL) and test A_{405} values. The best-fit curve should be plotted; usually a plot of reciprocal OD versus Aprotinin concentration gives a good correlation coefficient. Calculate the Aprotinin levels in the test samples from the standard curve. Plasma test samples giving Aprotinin levels higher than 250 KIU/mL should be assayed again, after further dilution (as appropriate), in acetone treated normal plasma. Other types of test samples, giving Aprotinin levels higher than 250KIU/mL should be assayed again after dilution in CTI+Buffer (5a). The Aprotinin values obtained should then be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS
Intra-assay CV: ≤5% at 50 KIU/mL. Detection limit: 12.5 KIU/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 dilutions may vary between different batches of this kit, owing to differences in the specific activity of some batches of reagents.

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