

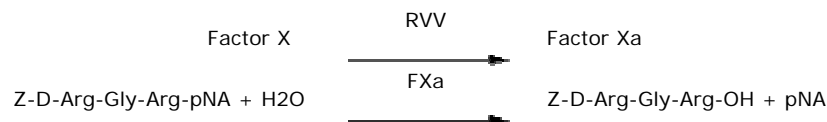


Factor X

Determination of factor X in plasma with S-2765

Measurement Principle

The method is based on a two-stage principle. In stage one, Factor X is activated in the presence of calcium to Factor Xa (FXa) using the activator Russell's Viper venom (RVV). In stage two, the generated FXa hydrolyses the chromogenic substrate Z-D-Arg-Gly-Arg-pNA (S-2765), thus liberating the chromophoric group pNA (p-nitroaniline). The colour is then read photometrically at 405 nm. The generated FXa (and thus the intensity of colour) is proportional to the FX activity of the sample.



Reagents

- S-2765, 25 mg Art. No. 82 14 13
Reconstitute the substrate S-2765 (MW: 714.6) with 20 ml sterile water.
- Russell's Viper Venom (RVV)
Prepare a solution of Russell's Viper Venom at a concentration of 0.087 mg/ml.
- CaCl₂
0.1 mol/l calcium chloride solution.
- Tris EDTA Buffer Art. No. 82 36 66
Dilute the buffer 1:10 with distilled water according to the insert sheet instructions.
- Normal Plasma
Calibrated, lyophilised or fresh frozen human plasma is used for the standardisation of the assay. A pooled normal plasma can be prepared by taking samples from 20 healthy donors. 10-30 ml citrate blood (9 vol blood and 1 vol 0.1 mol/l sodium citrate) from each donor is centrifuged at 2000 x g for 20 minutes at 15-25°C. The plasma is pooled and subsequently dispensed in small volumes, which are frozen rapidly at -20°C or below. To avoid low temperature activation of prekallikrein the plasma is kept at 15-25°C before use or freezing. Thawing of plasma should be performed at 37°C and then kept at 15-25°C until used.
- RVV + CaCl₂
Before use, mix 1 volume of RVV with 1 volume of CaCl₂. The mixture is stable for 48 hours at 2-8°C.
- Acetic acid 20%
Acetic acid is used as a stopper solution in the end-point method.

Specimen collection

Blood (9 vol) is mixed with 0.1 mol/l sodium citrate (1 vol) and centrifuged at 2000 x g for 20 minutes at 20-25°C. Storage: 1 week at 2-8°C or 3-4 months at -20°C.

Standard curve

FX %	Predilution		Final dilution	
	Normal plasma µl	Buffer µl	Predil. plasma µl	Buffer µl
0	-	-	-	1000
25	25	75	50	1000
50	50	50	50	1000
75	75	25	50	1000
100	-	-	50	1000
124	-	-	50	800

Method

Sample dilution	
Buffer	1000 µl
Test plasma or standard	50 µl
Mix	

Acid stopped method	A	B
Diluted sample	200 µl	50 µl
Incubate at 37°C	3-4 min	3-4 min
Substrate (37°C)	200 µl	50 µl
Mix and add within 30 sec		
RVV + CaCl ₂	200 µl	50 µl
Mix and incubate at 37°C	3 min	3 min
Acetic acid 20%	200 µl	50 µl

A= test tube method
B= microplate method

Sample blank activities should be determined and subtracted when analysing strongly coloured plasma, e.g. lipemic and hemolytic. The sample blanks are prepared by mixing the diluted sample, acetic acid 20% and water instead of the reagents (400 µl for test tubes and 100 µl for microplates). Read the absorbance of the samples and blanks at 405 nm. The colour is stable for at least four hours. When possible, use a dual wavelength mode with 490 nm as the reference wavelength.

Initial rate method:

When performing the initial rate method, transfer the microplate to a microplate reader immediately after the addition of RVV+CaCl₂ and read the change in A/min. The microplate reader must be pre-incubated at 37°C.

Calculation

Plot A or $\Delta A/\text{min}$ for the standards against their concentration of Factor X. Read the Factor X value for the corresponding A or $\Delta A/\text{min}$ of the unknown test sample from the standard curve.

Bibliography

1. Kiesel W et al. Factor X activating enzyme from Russell's Viper Venom; isolation and characterisation. *Biochemistry* 15, 4901-4905 (1976).
2. Lindhout MJ et al. Activation of decarboxyfactor X by a protein from Russell's Viper Venom. Purification and partial characterisation of activated decarboxyfactor X. *Biochem Biophys Acta* 533, 327-341, (1978).
3. Bergström K and Egberg N. Determination of vitamin K sensitive coagulation factors in plasma. Studies on three methods using synthetic chromogenic substrates. *Thromb Res* 12, 531-547 (1978).
4. Van Wijck EM et al. A rapid manual chromogenic factor X assay. *Thromb Res* 22, 681-686 (1981). Egberg N and Heedman PA. Simplified performance of amidolytic factor X assay. *Thromb Res* 25, 437-440 (1982).
5. Chabbat J et al. Aprotinin is a competitive inhibitor of the factor VIIa-tissue factor complex. *Thromb Res* 71, 205-215 (1993).
6. Mielicki WP and Gordon SG. Three stage chromogenic assay for the analysis of activation properties of factor X by cancer procoagulant. *Blood Coagul Fibrinol* 4, 441-446 (1993).
7. Koppaka V et al. Soluble phospholipids enhance factor Xa-catalyzed prothrombin activation in solution. *Biochemistry* 35, 7482-7491 (1996).
8. Riesbeck K et al. Human tissue factor pathway inhibitor fused to CD4 binds both FXa and TF/FVIIa at the cell surface. *Thromb Haemost* 78, 1488-1494 (1997).
9. Romisch J et al. Comparative in vitro investigation of prothrombin complex concentrates. *Semin Thromb Hemost* 24, 175-181 (1998)
10. Faria F, et al. A new factor Xa inhibitor (lefaxin) from the Haementeria depressa leec. *Thromb Haemost* 82, 1469-73 (1999)