

Prekallikrein Activator (PKA)

Determination of PKA in albumin and immunoglobulin preparations with S-2302

Measurement Principle

Prekallikrein (prekallikreinogenase) is activated to kallikrein by prekallikrein activator (PKA). The kallikrein formed catalyses the splitting of p-nitroaniline (pNA) from the substrate H-D-Pro-Phe-Arg-pNA (S-2302). The rate at which pNA is released is measured photometrically at 405 nm and can be followed on a recorder (initial rate method).

The correlation between the change in absorbance per minute (DA/min) and the prekallikrein activator concentration is linear between 0 and 51 IU/ml of prekallikrein activator.

The concentration of prekallikrein activator is calculated using an international standard.



Reagents

- S-2302, 25 mg Art. No. 82 03 40
Reconstitute the substrate S-2302 (MW: 611.6) with 6.8 ml of distilled water. Working solution: dilute one volume of the stock solution with nine volumes of the buffer (Reagent 2). The working solution is stable for 8 hours at 20-25°C.
- Tris Buffer, pH 7.8 (25°C)

Tris	6.1 g	(50 mmol/l)
NaCl	0.7 g	(12 mmol/l)
Distilled water	800 ml	

- Adjust the pH to 7.8 at 25°C by adding approximately 38 ml of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for six months at 2-8°C.
- Prekallikrein Activator
The 1 st International Standard 1984 (NIBSC, 82/530) contains 85 International Units per ampoule. Reconstitute with 1 ml of distilled water. Refer to PJ Kerry et al., Br J Haematol, 345-352, (1985) for more information on the standardisation of PKA.
- Prekallikrein fraction
A prekallikrein fraction is prepared according to the chromatography procedure described in the appendix. Check the quality of the prekallikrein according to paragraph J in the appendix before each test run. The prekallikrein solution is stable for at least one year at -70°C.

Sample

Albumin and immunoglobulin preparations.

Dilute the sample to a corresponding prekallikrein activator concentration of 10-40 IU/ml.

Standard curve

The 1 st International Standard has a PKA concentration of 85 IU/ml and is diluted as indicated in the table below.

PKA IU/ml	International Standard μl	Buffer μl
0	-	1000
10.2	120	880
20.0	235	765
34.9	410	590
50.2	590	410

Method

Initial rate method	
Step A for sample and standard	Sample Tube No. 1
Sample or standard	25 µl
Prekallikrein	100 µl
Mix and incubate at 37°C in capped tubes	45 min
Step B for sample and standard	Sample Tube No. 2
Substrate (37°C)	1000 µl
Mixture from tube No.1	25 µl
Mix	

Transfer sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) for measurement of the absorbance change for at least two minutes in a photometer at 405 nm and at 37°C. Immunoglobulin may occasionally contain significant kallikrein activities and thus a blank reading is necessary.

Step A for immunoglobulin blank	Blank Tube No. 1
Immunoglobulin	25 µl
Buffer (37°C)	100 µl
Mix	
Step B for immunoglobulin blank	Blank Tube No. 2
Substrate (37°C)	1000 µl
Mixture from tube No.1	25 µl
Mix	

Transfer sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) for measurement of the absorbance change for at least two minutes in a photometer at 405 nm and at 37°C.

Calculation

Calculate $\Delta A/\text{min}$. Perform the following calculation for the assay of prekallikrein activator in Immunoglobulin preparations:

$\Delta A/\text{min sample} - \Delta A/\text{min blank}$

Plot $\Delta A/\text{min}$ for the standards against their prekallikrein activator concentration. Calculate the prekallikrein activator concentration of the sample from the established standard curve.

Bibliography

1. Snape TJ et al. The assay of prekallikrein activator in human blood products. *Dev Biol Stand* 44, 115-120 (1979).
2. Kerry PJ et al. Standardisation of prekallikrein activator (PKA): the 1st International Standard for PKA. *Br J Haematol* 60, 345-352 (1985).
3. Briseid K et al. Part of prekallikrein removed from human plasma together with IgG-immunoblot experiments and functional tests. *Scand J Clin Lab Invest* 59, 55-63 (1999).
4. Briseid K et al. Removal of IgG from normal plasma and plasma from untreated patient active Crohn's disease-effect on levels of contact factors. *Scand J Clin Lab Invest* 60, 237-45 (2000).