

## 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 $\mu\text{l}$	Buffer $\mu\text{l}$
0	-	1000
10.2	120	880
20.0	235	765
34.9	410	590
50.2	590	410

## Method

Initial rate method	
<b>Step A for sample and standard</b>	<b>Sample Tube No. 1</b>
Sample or standard	25 µl
Prekallikrein	100 µl
Mix and incubate at 37°C in capped tubes	45 min
<b>Step B for sample and standard</b>	<b>Sample Tube No. 2</b>
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.

<b>Step A for immunoglobulin blank</b>	<b>Blank Tube No. 1</b>
Immunoglobulin	25 µl
Buffer (37°C)	100 µl
Mix	
<b>Step B for immunoglobulin blank</b>	<b>Blank Tube No. 2</b>
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).