Prekallikrein Activator (PKA)

Determination of PKA in albumin and immunoglobulin preparations with a chromogenic substrate

COA CHROM DIAGNOSTICA

Measurement Principle

Prekallikrein (prekininogenase) 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. 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 (ΔA/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.

$$\text{Prekallikrein} \xrightarrow{\text{PKA}} \text{Kallikrein}$$

$$\text{H-D-Pro-Phe-Arg-pNA + H2O} \xrightarrow{\text{Kallikrein}} \text{H-D-Pro-Phe-Arg-OH + pNA}$$

Reagents

1. Chromogenic substrate e.g. CS-31(02), 25 mg Art. No. 229031
   Reconstitute the substrate 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.
2. Tris Buffer, pH 7.8 Art. No. AR103A
3. Prekallikrein Activator
   E.g. the International Standard (NIBSC). Reconstitute with 1 ml of distilled water.
4. Prekallikrein pool fraction Art.No. COA0022

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 ΔA/min. Perform the following calculation for the assay of prekallikrein activator in Immunoglobulin preparations:
DA/min sample - ΔA/min blank
Plot ΔA/min for the standards against their prekallikrein activator concentration. Calculate the prekallikrein activator concentration of the sample from the established standard curve.

Bibliography

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