Plasmin and Streptokinase activated Plasmin Activity
Method Sheet

Single stage chromogenic method for the determination of Streptokinase activity

Principle

The method is provided as a one-stage assay for use in microplates, whereby a mixture of Glu-plasminogen and chromogenic substrate is added to the sample.
Streptokinase activates Glu-plasminogen to plasmin, the activity of which is determined with the chromogenic substrate S-2251.
As an option, the more sensitive chromogenic substrate S-2403 may be used.

Reagents

A. International Standard for Streptokinase

The standard is obtained from the WHO National Institute for Biological Standards and Controls, PO Box 1193, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QH, UK. Each ampoule is assigned an activity in International Units (IU).
The current standard is the 3rd Int Std 2001, with lot No 00/464.
Each freeze-dried ampoule shall be reconstituted to exactly 1000 IU/mL with buffer described in B below if not informed otherwise by NIBSC.
Optionally an in-house standard can be used as a secondary standard after careful calibration against the International Standard.

B. Potassium phosphate buffer, 0.15 mol/L, pH 7.5, 1% BSA

Dissolve 20.4 g of KH_2PO_4, 4.9 g NaOH and 10 g protease-free bovine serum albumin (BSA) in about 800 mL purified water. Adjust pH to 7.5 with 1 mol/L NaOH and make up the volume to 1000 mL with water. Filter the buffer and keep it at +4C. The stability is one week at +4C.

C. Plasminogen, 1.5 mg.

This bioreagent, containing purified human Glu-plasminogen, is obtained from Chromogenix, Milan, Italy, or through its distributors.
Each vial shall be reconstituted with 20 mL of buffer B. The solution shall be used immediately. Excess amounts may be frozen once and stored preferably below -20C. The stability of the frozen material is one month.

D. Chromogenic substrate S-2251, 25 mg.

The reagent is a chromogenic substrate for plasmin and is obtained from Chromogenix, Milan, Italy, or through its distributors.
It contains 25 mg of lyophilized H-D-Val-Leu-Lys-pNA and also mannitol (bulking agent).
Each ampoule shall be reconstituted with 20 mL of water. The solution is stable for 6 months at +4C, provided no bacterial contamination occurs.
Optionally, the plasmin substrate S-2403 (pyro-Glu-Phe-Lys-pNA) may be used.

E. Working reagent of plasminogen and S-2251.

Mix one volume of plasminogen (C) with one volume of S-2251 solution (D). Use the solution immediately.

F. Citrate buffer, 1 mol/L, pH 3.0 (Stop reagent)

Dissolve 30 g of citric acid $C_6H_8O_7$ x 2 H_2O and 15 g of sodium citrate, $C_6H_5Na_3O_7$ x H_2O in 200 mL of water.
The solution is stable at 20-25C for two weeks.

Method

1. Dilution of samples and standard

If the International Standard is used, it should be reconstituted to exactly 1000 IU/mL with room tempered buffer B (see A). An in-house standard should be prediluted to exactly 1000 IU/mL with the same buffer. Samples should be prediluted to roughly 1000 IU/mL with the same buffer.

Prepare secondary dilutions of samples and standards from the 1000 IU/mL by performing dilutions 1:50, 1:100 and 1:200 with room tempered buffer B.

2. Performance

2.1 Overview of method

Samples / standards	25 µL
Working Reagent of Plg/S-2251	100 µL
Mix and incubate 15-20 min at 20-25C	
Stop Reagent	50 µL

2.2 Detailed procedure

Load 25 □L of samples/standards to the microplate wells. Add duplicates of all dilutions and load one set of standards (duplicates) before the sample wells and one set of standards (duplicates) in wells located after the samples.

Prepare the Working Reagent as described in E. Add 100 □L of working reagent to all wells. In case an automated equipment is used, prepare a sufficient volume of working reagent to compensate for the dead volume of the instrument.

Incubate at 20-25C for 15-20 min to reach an absorbance of 0.80 - 1.20 for the secondary dilution 1:50.

Add 50 □L of Stop Reagent to all wells. Read the plate at 405 nm, using 490 nm as reference wavelength.

Make sure that no contamination occurs at any step during the assay.

2.3. Calculation

The results are preferably evaluated as a parallel line assay relating ln A405-490 to ln activity.

2.4 Linearity

The method is linear in the range 0 - 40 IU/mL.

2.5 Precision

Inter assay precision is < 4% RSD (relative standard deviation)

2.6 Limitations

Samples with less than 100 IU/mL (after predilution) rather than the targeted range of 1000 IU/mL may not fulfill requirements on parallelism vs the standard and/or linearity. In such cases the assay should be repeated with less predilution of the sample.