Recombinant Streptokinase Bulk Solution (IPC Draft with suggestions from NIB and participants)

Recombinant Streptokinase Bulk Solution is a fibrinolytic enzyme produced by a method based on recombinant DNA technology using bacteria or suitable genetically engineered host cells.

Streptokinase Bulk Solution has a potency of not less than 600 IU per microgram of nitrogen.

Category. Thrombolytic agent

Production

If intended for use in the manufacture of parenteral preparations, the method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Host cell-derived proteins (HCP)

The limits are not more than 100 ppm.

Host cell and vector-derived DNA

The limits are not more than 10ng/dose (1 dose = 1.5MIU).

Description. A clear, colourless to slightly yellowish liquid.

Identification

A. Clot Lysis method: Place 0.5 ml of citrated human plasma in a haemolysis tube maintained in a water-bath at 37°C. Add 0.1 ml of a solution of the contents of the container containing 10 000 IU of streptokinase activity per millilitre in phosphate buffer pH 7.2 and 0.1 ml of a solution of human thrombin containing 20 IU/ml in phosphate buffer pH 7.2. Mix immediately. A clot forms and lyses within 30 min. Repeat the procedure using citrated bovine plasma. The clot does not lyse within 1 hour.

B. Determine by western blotting method, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under reducing condition

Run a 12% SDS PAGE gel after loading the lanes with preparation under examination equivalent to 10-20 µg Recombinant Streptokinase Bulk Solution and *bovine serum albumin* as negative control. Set up the transfer blotting apparatus. Develop the blot with suitable developer. The end result is the development of colour only in the sample protein lane (Recombinant Streptokinase main band) which is identified & confirmed by the corresponding antibody.

C. Determine purity by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under reducing condition.

SDS polyacrylamide gel electrophoresis the mobility depends on the protein's molecular size. Run a 10-12% resolving gel mix and 5% stacking gel after loading the lanes with 10-20 µg equivalents Recombinant Streptokinase Bulk Solution. Stain the gel with coomassie blue and destain. Document the gel for future reference.

No artefacts are visualized in the sample protein lane. Protein band intensity is appropriate to the protein load. Main band same position as standard & in line wrt the molecular weight markers. Main band ≥ 97% of total integrated bands.

D. Determine purity by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)(2.4.12) under non-reducing condition.

Native polyacrylamide gel electrophoresis the mobility depends on both the protein's charge and its hydrodynamic size. The electric charge driving the electrophoresis is governed by the intrinsic charge on the protein at the pH of the running buffer.

Run 8% native resolving gel after loading the lanes with 10-20 µg equivalents Recombinant Streptokinase Bulk Solution. Stain the gel with coomassie blue and destain. Document the gel for future reference.

No artefacts are visualized in the sample protein lane. Protein band intensity is appropriate to the protein load. Main band same position as standard & main band ≥ 98% of total integrated bands.

E. Determine pI by isoelectric focussing polyacrylamide gel electrophoresis (PAGE) (2.4.33) under non-reducing condition.

Isoelectric focusing is a method of electrophoresis that separates proteins according to their net charge (pI). Separation is carried out in poly acrylamide gel which contains the mixture of amphoteric electrolytes called poly ampholytes (small multicharged polymers that have many pI values).

Run 6% resolving gel mix with ampholytes (3-10, Broad range pI) after loading the lanes with 10-20 µg equivalents Recombinant Streptokinase Bulk Solution. Stain the gel with coomassie blue and destain. Document the gel for future reference.

No artefacts are visualized in the sample protein lane. Protein band intensity is appropriate to the protein load. The sample should have single main band at R*f* position pI 5.5-6.1 is observed.

Tests

pH. 6.8 to 7.5, determined in a solution prepared by diluting the preparation under examination in *carbon dioxide-free water* to produce a solution containing at least 10^5 IU of streptokinase activity per millilitre.

Protein. Determine by nitrogen content (2.3.30). 1 mg of N is equivalent to 6.25 mg of protein.

Assay

Potency

The potency of streptokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of streptokinase calibrated in International Units; the formation of plasmin is determined using a suitable chromogenic substrate.

The International Unit is the activity of a stated amount of the International Standard for streptokinase. The equivalence in International Units of the International Standard is stated by the regulatory authority.

Reference and test solutions: Prepare 2 independent series of 4 dilutions of each of the substance under examination and of the reference preparation of Streptokinase in 0.5 M *tris (hydroxymethyl) aminomethane sodium chloride buffer pH 7.4 containing 1% w/v Human Serum Albumin*, in the range of 0.5, 1.0, 2.0 & 4.0 IU/ml.. Prepare and maintain all solutions at 37ºC.

Substrate solution: Mix 1.0 ml of *tris (hydroxymethyl) aminomethane buffer pH 7.4* with 1.0 ml of 3mM chromophore substrate. Add 5 µl of a 10 per cent w/v solution of *polysorbate 20 or*10% Tween 20. Keep at 37ºC in a water-bath. Immediately before commencing the activation assay, add 45 µl of a 1 mg per ml solution of human plasminogen.

Analyse each streptokinase dilution, maintained at 37ºC, in duplicate. Initiate the activation reaction by adding 60 µl of each dilution to 40 µl of substrate solution. For blank wells, use 60 µl of *tris (hydroxymethyl) aminomethane sodium chloride buffer solution pH 7.4* instead of the reference and test solutions. Allow the reaction to proceed at 37ºC for 20 minutes and read the absorbance at 405 nm. If a suitable thermostated plate reader is available, this may be used to monitor the reaction. Alternatively, it may be necessary to stop the reaction after 20 minutes using 50 µl of a 50 per cent v/v solution of *glacial acetic acid*. Best results are obtained when the absorbance for the highest streptokinase concentration is between 0.1 and 0.2 (after blank subtraction). If necessary, adjust the time of incubation in order to reach this range of absorbances.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of the reference preparation of streptokinase and calculate the potency of the substance under examination using the usual statistical methods for parallel-line assays.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

Streptokinase Bulk Solution intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.02 Endotoxin Unit per 100 IU of streptokinase activity.

Storage. Store protected from light and at a temperature of about - 20°. If it is intended for the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labeling. The label states (1) the number of International Units of streptokinase activity per mg, calculated on the dried basis; (2) the name and quantity of any added substance; (3) where applicable, that the substance is free from bacterial endotoxins; (4) where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

Recombinant Streptokinase Injection (As proposed by NIB and accepted by participants)

Recombinant Streptokinase Injection produced by recombinant DNA technology using bacteria or suitable genetically engineered host cells with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile water for injection, immediately before use.

Description: A white powder or a white, friable solid, hygroscopic.

Usual strengths: 50,000 Units; 100,000 Units; 7,50,000 Units; 15,00,000 Units.

Identification

A. Clot Lysis method: Place 0.5 ml of citrated human plasma in a haemolysis tube maintained in a water-bath at 37 °C. Add 0.1 ml of a solution of the contents of the container containing 10 000 IU of streptokinase activity per millilitre in phosphate buffer pH 7.2 and 0.1 ml of a solution of human thrombin containing 20 IU/ml in phosphate buffer pH 7.2. Mix immediately. A clot forms and lyses within 30 min. Repeat the procedure using citrated bovine plasma. The clot does not lyse within 1 hour.

Tests

pH. 6.8 to 7.5, determined in a solution prepared by diluting the preparation under examination in water for injection to produce a solution containing 5000 IU of streptokinase activity per millilitre.

Assay

Potency

The potency of streptokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of streptokinase calibrated in International Units; the formation of plasmin is determined using a suitable chromogenic substrate.

The International Unit is the activity of a stated amount of the International Standard for streptokinase. The equivalence in International Units of the International Standard is stated by the regulatory authority.

Reference and test solutions: Prepare 2 independent series of 4 dilutions of each of the substance under examination and of the reference preparation of streptokinase in 0.5 M T*ris (hydroxymethyl) aminomethane sodium chloride buffer pH 7.44 containing 1% w/v Human Serum Albumin*, in the range of 0.5, 1.0, 2.0 and 4.0 IU per ml. Prepare and maintain all solutions at 37º C.

Substrate solution: Mix 1.0 ml of 0.5 M T*ris (hydroxymethyl) aminomethane buffer pH 7.4* with 1.0 ml of 3 mM chromophore substrate. Add 5 µl of a 10 per cent w/v solution of *polysorbate 20 or Tween 20*. Keep at 37º C in a water-bath. Immediately before commencing the activation assay, add 45 µl of a 1 mg per ml solution of human plasminogen.

Analyse each streptokinase dilution, maintained at 37ºC, in duplicate. Initiate the activation reaction by adding 60 µl of each dilution to 40 µl of substrate solution. For blank wells, use 60 µl of *tris (hydroxymethyl) aminomethane sodium chloride buffer solution pH 7.4* instead of the reference and test solutions. Allow the reaction to proceed at 37º C for 20 minutes and read the absorbance at 405 nm. If a suitable thermostated plate reader is available, this may be used to monitor the reaction. Alternatively, it may be necessary to stop the reaction after 20 minutes using 50 µl of a 50 per cent v/v solution of *glacial acetic acid*. Best results are obtained when the absorbance for the highest streptokinase concentration is between 0.1 and 0.2 (after blank subtraction). If necessary, adjust the time of incubation in order to reach this range of absorbances.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of the reference preparation of streptokinase and calculate the potency of the substance under examination using the usual statistical methods for parallel-line assays.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

Bacterial endotoxins (2.2.3). Not more than 23.33 Endotoxin Unit per 10000 IU/ ml of streptokinase activity.

Sterility: (2.2.11) Complies with the test for sterility.

Abnormal toxicity: (2.2.1). Determine by Method.A, using a solution containing 50,000 Units in 0.5 ml of water for injection administered in 15 to 20 minutes.

Tests performed for parenterals (Under Powder for injection) includes Particulate matter, Clarity.

Storage. Store in sealed containers, protected from light in a refrigerator (2 º to 8ºC). The containers should be sterile and sealed so as to exclude microorganisms. Under these conditions the contents may be expected to retain their potency for 2 years.

Labeling. The label states the total number of units of Streptokinase activity contained in it.

Discrepancies with regard to Streptokinase Monographs in I.P.2010 and Suggestions made by the participants.

Test Parameter	**Streptokinase (Page 2157)**	**Streptokinase Bulk solution (Page 2617)**	**Streptokinase injection (Page 2159)**	**Meeting out come/ finalized draft**
Identification				
a. Clot lysis	Canine or rabbit plasma	Not mentioned	Canine or rabbit plasma	May be specified as common to all.
	Thrombin	Thrombin RS	Thrombin	May be specified as Thrombin to all.
b. DID	Mixed Barbitone buffer	Barbitone buffer	Mixed Barbitone buffer	May be specified as common to all.
pH	Boiled and cooled water	Carbon dioxide free water	Freshly constituted injection	May be specified as common to all.
Streptodornase activity	Calculation formula (A1-A2)< 0.5(A3+A4)-A2	Calculation formula (A3+A4)-(A1+A2)< (A5+A6+A7+A8)/ 2 -(A3+A4)	Calculation formula (A1-A2)< 0.5(A3+A4)-A2	May be changed to (A2- A1)< 0.5(A3+A4)-A2 for Streptokinase and Streptokinase injections.
Streptolysin activity	The absorbance of the supernatant liquid at about 550 nm is not more than 1.5 times the absorbance of the test solution.	The absorbance of the test solution is not more than 50 per cent than that of the reference solution.	The absorbance of the supernatant liquid at about 550 nm is not more than 1.5 times the absorbance of the test solution.	May be specified as common to all.
Assay/Potency	Clot lysis method	Chromogenic method	Clot lysis method	May be specified Chromogenic method to all.
			Determine on the mixed contents of ten containers.	May be specified as three containers.
Abnormal toxicity	Yes	Yes	Specified under power for injections	May be given as parameter in the list of tests to be performed on the product.

Along with above suggestions some of these may be included in the monographs

- Identification of Streptokinase, Clot lysis **and**/**or** Double Immuno Diffusion can be suggested.
- Assay/potency **International Reference Standard for Streptokinase** can be replaced against 1st International reference standard 1964.
- Streptokinase activity- The potency is not less than 510 IU/microgram of Nitrogen. can be specified instead of 600 IU given in Streptokinase (Page 2157, IP2010 and proposed monograph of r- Streptokinase bulk solution)