KRISHZYME® Streptokinase / Streptodornase Assay Kit

REF : KBBA15

Ver 2.1

RUO

Chromogenic Assay for Quantitative determination of Streptokinase and Streptodornase in Biological Samples

RUO	For Research Use Only	REF	Catalog Number
	Stor	LOT	Batch Code
	Manufactured By		Bio al Risk
	Exp te		Co Operating Instructions

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REF KBBA15 100 **tests**

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Introduction:

A mixture of the enzymes (streptokinase and streptodornase) is produced by hemolytic streptococci. It is used topically on surface lesions and by instillation in closed body cavities to remove clotted blood or fibrinous or purulent accumulations. Streptokinase-streptodornase (OSS) has both anti-inflammatory and useful hematologic effects. It is also used as a skin test antigen in evaluating generalized cell-mediated immunodeficiency. A rapid rate of synthesis is inferred from the restoration of normal plasma concentrations within 12-24 hours of depletion during thrombolytic therapy with streptokinase.

Intended use:

KRISHZYME® Streptokinase / Streptodornase Assay Kit are used for accurate measurement of Streptokinase and Streptodornase in biological samples.

Principle:

The chromogenic method is a kinetic method using a chromogenic substrate where Streptokinase converts plasminogen to plasmin in solution, and in the absence of fibrin. In this method, a given concentration of Streptokinase results in linear generation of active plasmin and accelerating hydrolysis of substrate hence results in the optical density (OD) of the solution increasing linearly at 405 nm.

Streptodornase is a DNAse enzyme and the assay method involved incubation for a set time at 37°C with a solution of calf thymus DNA. The reaction was stopped by precipitation with perchloric acid and soluble nucleotides were quantitated by measuring absorbance at 260 nm.

Materials provided in Kit:

1. Streptokinase Standard 300 IU/ml (lyophilized) - 2 vials
2. Chromogenic Substrate (Frozen) - 2 vials
3. Human Plasminogen (Frozen) - 2 vials
4. Calf Thymus DNA (Frozen) - 1 vial
5. Streptodornase Standard 200 IU/ml (Frozen) - 2 vials
6. Streptokinase Sample/Standard Diluent - 15 ml
7. Streptodornase Sample/Standard Diluent - 25 ml
8. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 405 nm and 260nm.
2. Microplates
3. Eppendorf tubes
4. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.
5. Deionized (DI) water.
6. Glacial acetic acid
7. Perchloric acid
8. Wash bottle or automated microplate washer.
9. Semi-Log graph paper or software for data analysis.
10. Tubes to prepare standard/sample dilutions.
11. Timer.
12. Absorbent paper.

Storage Information:

1. Store Chromogenic Substrate, Human plasminogen, Calf Thymus DNA and Streptodornase at -20°C.
2. Store the Standard after reconstitution at -20°C. Rest all kit components shall be stored at 2-8°C.

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Reagent Preparation:

1. **Streptokinase Standard (300 IU/ml):**
   Reconstitution: Add 1 ml Distilled water in vial (300 IU/ml).
   Prepare standard concentration as shown in **Table 1** by using standard diluent.

2. **Chromogenic Substrate solution:**
   Add 2.11 ml Distilled water in vial and it will become a ready to use solution.

3. **Plasminogen:**
   Add 2.472 ml Distilled water in vial and it will become a ready to use solution.

4. **Stop Solution for Streptokinase:** ***(not provided in kit)***
   Prepare Glacial Acetic Acid 4.2 ml of Glacial Acetic Acid + 5.8 ml of Distilled Water.

5. **Calf Thymus DNA:**
   Dilution: Add 150 ul of **Calf Thymus DNA** to 350 ul of Sample/Standard diluent (for Streptodornase) to make final volume to 500 ul.

6. **Streptodornase Standard (200 IU/ml):**
   Prepare standard concentration as shown in **Table 3** by using Sample/Standard diluent (for Streptodornase).

7. **Stop solution for Streptodornase:** ***(not provided in kit)***
   Add 10.5 ml of 70% perchloric acid in 250 ml of distilled water.

***For Streptokinase*:**
Standard Preparation:
Reconstitute the lyophilized vial as mentioned in reagent preparation.
Main stock (MS): 300 IU/ml

Table 1:

Std. No	Standard concentration (IU/ml)	Standard volume (ul)	Standard Diluent (ul)	Total volume (ul)
S1	160	213.3 ul of MS	186.7	400
S2	80	200 ul of S1	200	400
S3	40	200 ul of S2	200	400
S0	0	0 ul	200	200

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. Run a standard curve as per chart details.

Table 2:

STK Conc IU/ml	0	40	80	160
Time (mins)	Abs 0	Abs 1	Abs 2	Abs 3
0				
10				
20				
30				
40				
50				
60				
70				
80				
90				

2. For blank: Add **100 ul of Sample diluent** and **50 ul of Chromogenic substrate** to the plate and incubate at **37°C** for **5 minutes**.

3. For Standards/Samples: Add **50 ul of Sample/ Standard** and **50 ul of Chromogenic substrate** to the plate and incubate at **37°C** for **5 minutes**.

4. Add **50 ul of prepared plasminogen** solution to respective **Sample/ Standard** wells, seal plate.

5. Incubate at **37°C** for **90 minutes** measuring OD at **405 nm** with 10 mins regular intervals up to 90 mins as shown in **Table 2**.

6. At 90 mins, add **100 ul of Stop Solution** to each well.

7. Read absorbance at **405 nm** within 15 minutes of stopping reaction.

For Streptodornase:
Standard Preparation:
Reconstitute the lyophilized vial as mentioned in reagent preparation.
Main Stock (MS): 200 IU/ml

Table 3:

Sr. No	Standard Concentration (IU/ml)	Standard Volume (ul)	Standard Diluent (ul)	Total Volume (ul)
1	200	800	0	800
2	100	400 ul of 1	400	800
3	50	400 ul of 2	400	800
4	25	400 ul of 3	400	800
5	12.5	400 ul of 4	400	800
6	6.25	400 ul of 5	400	800
7	3.125	400 ul of 6	400	800
8	0	0	400	400

Assay Procedure:

Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

(A) For Standards / Samples:

1. Pre equilibrate **Streptodornase Standard and Calf Thymus solution for 5 mins at 37°C.**
2. Add **125 ul of Calf Thymus solution** to 2.0 ml eppendorf tubes.
3. Add **125 ul of Streptodornase Standard** to respective tubes and swirl the tube gently to mix both the solutions (Do not vortex the mixture).
4. **Incubate at 37°C for 60 minutes.**
5. Add **1000 ul of pre-chilled Stop Solution** (**Perchloric Acid** Solution) to each tube and vortex vigorously.
6. Transfer the tubes on ice and incubate for 10 minutes.
7. Centrifuge the tubes for 15 minutes at 13500 rpm at 4°C.
8. Pipette out **200 ul** of supernatant and transfer to microwells and measure absorbance value at 260 nm.

(B) For blank:

1. **Add 1000 ul of Perchloric Acid** solution to 2.0 ml eppendorf tubes.
2. Add **125 ul of Calf Thymus Solution and 125 ul of** Streptodornase Sample/Standard diluent to respective tubes and mix gently.
3. Centrifuge the tubes for 15 minutes at 13500 rpm at 4°C.
4. Pipette out **200 ul** supernatant and transfer to microwell and measure absorbance value at 260 nm.

Calculation of Results:

For each series, calculate the regression of the absorbance against log concentration of the sample solutions and the standard solutions. Calculate the potency of the Streptokinase and Streptodornase in IU using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the Streptokinase and Streptodornase activity of the sample in mg.

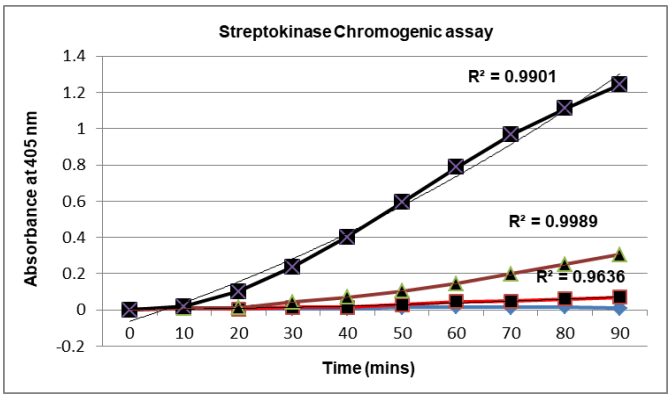
Standard and Test Samples being serially diluted should pass the test for linearity and parallelism as the interpretation is done by extrapolating the data. We have used proprietary MS Excel software for the same based on DJ Finney algorithm.

Typical Data (representative only)
Streptokinase Assay

STK Conc. IU/ml	0	40	80	160
Time (mins)	Corrected Abs 0	Corrected Abs 1	Corrected Abs 2	Corrected Abs 3
0	0	0	0	0
10	0.0095	0.0115	0.0081	0.0198
20	0.0055	0.0047	0.0121	0.1003
30	0.0078	0.015	0.0421	0.2367
40	0.0067	0.0128	0.0705	0.4025
50	0.0138	0.0282	0.1047	0.5933
60	0.0171	0.0453	0.1438	0.7879
70	0.0142	0.0476	0.197	0.9658

80	0.0169	0.0591	0.2504	1.1124
90	0.0086	0.0697	0.305	1.2424

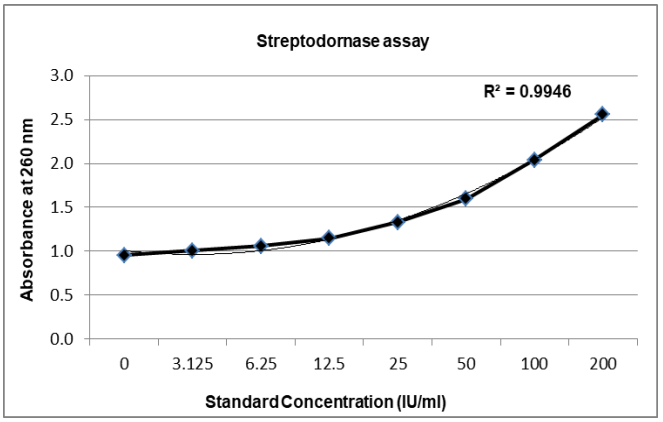
Typical Graph (representative only)
Streptokinase Assay



Typical Data (representative only)
Streptodornase Assay:

Standard Conc (IU/ml)	Abs 1	Abs 2	Mean Abs
0	0.956	0.952	0.954
3.125	1.003	1.007	1.005
6.25	1.063	1.046	1.055
12.5	1.141	1.152	1.146
25	1.320	1.341	1.331
50	1.599	1.590	1.595
100	2.027	2.051	2.039
200	2.570	2.546	2.558

Typical Graph (representative only)
Streptodornase Assay:



Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is For Research Use only and Not For Diagnostic Use**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from **human body fluids** or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.



- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

  In any case GLP should be applied with all general and individual regulations to the use of this kit**.**



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