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ab273313 Streptokinase Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273313
(use https://www.abcam.cn/ab273313 for china, or
https://www.abcam.co.jp/ab273313 for Japan)

For the determination of Streptokinase activity in serum samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Streptokinase Activity Assay Kit (Colorimetric) (ab273313) can be used to evaluate Streptokinase (SK) produced in bulk culture and its effect on plasminogen activation in serum. The assay is simple, easy to perform and can be completed in 3 easy steps. The assay can detect as little as 3 mU of Streptokinase Activity in less than 60 mins.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells, remembering to incubate plate for 5 mins at 37°C before adding prepared SK to Positive Control.

Measure the absorbance in a kinetic mode at 1 min intervals for 60 mins at 37°C.

Determine SK activity using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
SK Assay Buffer	6.5 mL	-20°C
SK Substrate Buffer	3.5 mL	-20°C
SK Substrate	850 µL	-20°C
Plasminogen	1 vial	-20°C
pNA Standard	20 µL	-20°C
Streptokinase (Lyophilized)	1 vial	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Clear 96-well microplate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Distilled Water

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Allow kit contents to thaw to RT.

9.1 SK Assay Buffer:

Store at -20°C. Immediately before use, bring to RT.

9.2 SK Substrate Buffer:

Store at -20°C. Immediately before use, bring to RT.

9.3 SK Substrate:

Thaw and aliquot into amber vials. Protect from light and store at -20°C.

9.4 Plasminogen:

Resuspend in 200 µl of dH_2O to prepare Plasminogen stock solution. Aliquot and store -20°C for up to 6 months. Avoid repeated freeze thaw cycles.

9.5 pNA Standard:

Thaw and aliquot into amber vials. Protect from light and store at -20°C.

9.6 Streptokinase (Lyophilized):

Centrifuge briefly. Reconstitute the vial in 44 µl dH_2O to prepare Streptokinase stock solution. Allow the enzyme to rehydrate for 10 mins before use. Aliquot and store at -20°C for long term storage. This will be used as the Positive Control for the experiment.

10. Sample Preparation

– We recommend evaluating in-house preparations of recombinant Streptokinase (rSK) at multiple dilutions.

10.1 Dilute rSK at 1:10, 1:100 and 1:1000 dilutions.
10.2 Prepare 1:10 dilution of rSK Sample by adding 20 µl of rSK into 180 µl of SK Assay Buffer.
10.3 Add 20 µl of 1:10 diluted rSK into 180 µl of SK Assay Buffer to prepare 1:100 dilution of rSK.
10.4 Add 20 µl of 1:100 diluted rSK into 180 µl of SK Assay Buffer to prepare 1:1000 dilution of rSK.
10.5 Add 40 µl of each rSK Sample dilution per well labeled as Sample and Sample Background respectively.
10.6 Add 40 µl of SK Assay Buffer to the Reagent Background well and 100 µl of SK Assay Buffer to the Blank well respectively.
10.7 Positive Control: Prepare a well labeled as Positive Control.

ΔNote: For Unknown Samples, we suggest doing a pilot experiment and testing several doses to ensure the readings are within the range of the Standard Curve.

ΔNote: For Samples having high background, prepare a parallel Sample well labeled as Sample Background.

ΔNote: Tissue and cell lysates can also be evaluated for Streptokinase Activity along with the in-house preparations of rSK. Simply prepare two wells for each Sample type containing 40 µl rSK Sample or lysate and label them as Sample and Sample Background well respectively.

11. Standard Curve

11.1 Add 5 µl of 0.1 M pNA Standard into 95 µl SK Assay Buffer to prepare 5 mM pNA Standard solution.

11.2 Add 0, 2, 4, 6, 8, 10 µl of 5 mM pNA Standard into each well individually.

11.3 Adjust the volume to 100 µl/well with SK Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard.

Standard #	5 µM pNA - Standard (µL)	SK Assay Buffer (µL)	pNA (nmol/well)
1	10	90	50
2	8	92	40
3	6	94	30
4	4	96	20
5	2	98	10
6	0	100	0

12. Assay Procedure

Reaction mix:

12.1 Prepare SK Substrate Buffer working solution by diluting SK Substrate Buffer 1:1 with dH_2O.

12.2 Prepare SK Substrate working solution by adding 11 µl of SK Substrate to 4 µl of dH_2O.

12.3 Prepare Plasminogen working solution by adding 2 µl of stock Plasminogen solution to 18 µl of SK Assay Buffer.

12.4 Mix enough reagents for the number of assays to be performed. For each well, prepare 60 µl of Reaction Mix containing:

	Reaction Mix (µl)	Background Control Mix (µl)
SK Substrate Buffer working solution	40	50
SK Substrate working solution	10	---
Plasminogen working solution	10	10

12.5 Mix well. Add 60 µl Reaction Mix to Positive Control, Reagent Background and Sample wells.

12.6 Add 60 µl Background Control Mix to the Sample Background well(s). Mix well.

12.7 **Incubate the plate in a pre-heated plate reader for 5 mins at 37°C.**

12.8 Upon removal of the plate, add 4 µl of the reconstituted Streptokinase stock solution and 36 µl SK Assay Buffer into the Positive Control well.

Positive Control: 60 µl Reaction Mix, 4 µl of the reconstituted Streptokinase stock solution and 36 µl of SK Assay Buffer.
Reagent Background: 40 µl of SK Assay Buffer and 60 µl.
Reaction Mix Sample: 40 µl Sample and 60 µl Reaction Mix.
Sample Background: 40 µl Sample, 60 µl Background Control Mix.
Blank: 100 µl SK Assay Buffer.

12.9 Read the absorbance in a kinetic mode at OD 405 nm at 1 min intervals for 60 mins at 37°C. The pNA Standard Curve

can be read in kinetic or endpoint mode (at the end of the incubation time).

13. Calculations

13.1 Subtract 0 Standard reading from all Standard readings.
13.2 Plot the pNA Standard Curve.
13.3 If the Sample Background reading is significant, subtract the Sample Background reading from its paired Sample readings.
13.4 Choose any two time points within the linear portion of the curve (t1 and t2) for each Sample.
13.5 Apply the corrected Sample readings to the pNA Standard Curve to get B nmol of pNA generated during the reaction time ($\Delta t = t2-t1$).
13.6 Calculate the Sample Streptokinase Activity as shown below:

$$Sample\ Streptokinase\ \ activity\ = \frac{B}{(\Delta t\ x\ V)}\ \times D\ \ (nmol/ml)$$

B = pNA generated in the Sample well (nmol)
$\Delta t = (t2-t1)$ (min)
V = Sample volume added into the reaction well (ml)
D = Sample dilution factor

Unit definition:

One Unit of Streptokinase is the amount of enzyme that generates 1 µmole of pNA per min at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

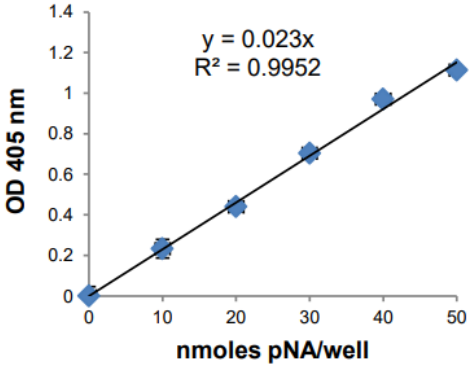

Figure 1. pNA Standard Curve with 0-50 nmoles pNA/well.

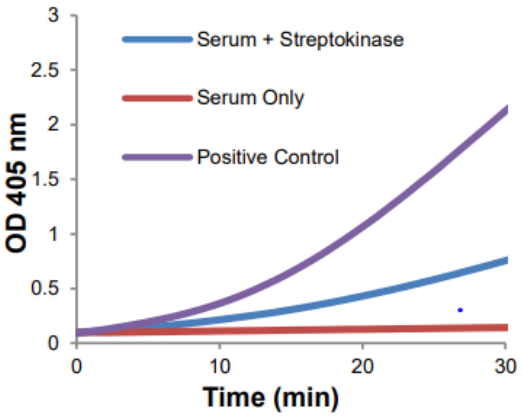

Figure 2. The graph shows the increase in OD values generated when Streptokinase is added to serum (indicating the increased level of plasmin activated by Streptokinase) and compared to Serum Background (Streptokinase without serum). Streptokinase is the positive control.

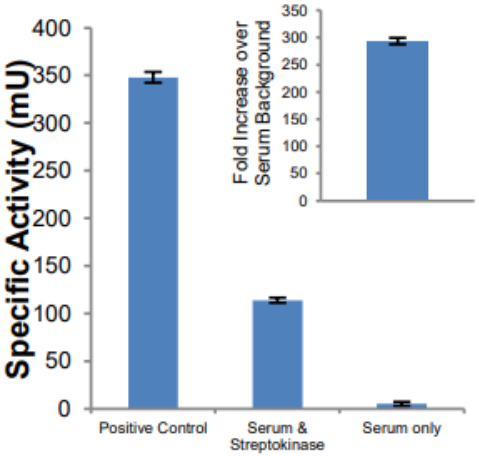

Figure 3. Fold increase in absorbance with the addition of Streptokinase to serum-off-the-clot over Background (inset).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)