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Streptokinase Activity Assay Kit (Colorimetric)

rev 06/21

(Catalog # K2008-100, 100 assays; Store at -20 °C)

I. Introduction:

Human Streptokinase is a therapeutic drug and is used as a fibrinolytic agent to treat patients with acute myocardial infarction. Streptokinase (SK) is a plasminogen activator, which works by binding to plasminogen to form a catalytically active streptokinase-plasminogen enzymatic complex (Plg-SK) thereby converting plasminogen to plasmin. In nature, Streptokinase is produced by Group A and Group C Streptococci. SK helps Streptococci in hijacking plasminogen in the human fibrinolytic system thereby dissolving the host fibrin barriers and facilitating bacterial spreading. SK is listed in the World Health Organization (WHO) Model List of essential medicines and is a leading fibrinolytic agent in the treatment of thromboembolic conditions. It is cost-effective and is easy to produce. **BioVision's Streptokinase Activity Assay Kit** can be used to evaluate Streptokinase 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 min.

The overall reaction is as follows:

Plasminogen + SK ⟶ Plg-SK ⟶ SK + Plasmin

Plasmin + SK Substrate ⟷ Plasmin-SK Substrate ⟷ Plasmin + *p*NA (Absorbance 405 nm)

II. Application:

Determination of recombinant Streptokinase activity in bulk preparation for comparison with the in-house bulk-preparation.

III. Sample Type:

Serum off the clot can be evaluated for increased plasmin activity with the addition of Streptokinase.

IV. Kit Contents:

Components	K2008-100	Cap Code	Part Number
SK Assay Buffer	6.5 ml	WM	K2008-100-1
SK Substrate Buffer	3.5 ml	Amber/NM	K2008-100-2
SK Substrate	850 µl	Blue	K2008-100-3
Plasminogen	1 vial	Red	K2008-100-4
*p*NA Standard	20 µl	Yellow	K2008-100-5
Streptokinase	1 vial	Orange	K2008-100-6

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Allow kit contents to thaw to room temperature (RT).

- **SK Assay Buffer, SK Substrate Buffer:** Store at -20 °C. Immediately before use, bring to RT.
- **SK Substrate:** Thaw and aliquot into amber vials. Protect from light and store at -20 °C.
- **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.
- ***p*NA Standard (0.1 M):** Thaw and aliquot into amber vials. Protect from light and store at -20 °C.
- **Streptokinase:** Centrifuge briefly. Reconstitute the vial in 44 µl dH_2O to prepare Streptokinase stock solution. Allow the enzyme to rehydrate for 10 min before use. Aliquot and store at -20 °C for long term storage. This will be used as the **Positive Control** for the experiment.

VII. Streptokinase Activity Assay Protocol:

1. Sample Preparation:

Evaluation of recombinant Streptokinase (rSK) Sample: We recommend evaluating in-house preparations of rSK at multiple dilutions. For example, dilute rSK at 1:10, 1:100 and 1:1000 dilutions. Prepare 1:10 dilution of rSK Sample by adding 20 µl of rSK into 180 µl of SK Assay Buffer. Add 20 µl of 1:10 diluted rSK into 180 µl of SK Assay Buffer to prepare 1:100 dilution of rSK. Add 20 µl of 1:100 diluted rSK into 180 µl of SK Assay Buffer to prepare 1:1000 dilution of rSK. Add 40 µl of each rSK Sample dilution per well labeled as **Sample** and "**Sample Background**" respectively.

Add 40 µl of SK Assay Buffer to the "**Reagent Background"** well and 100 µl of SK Assay Buffer to the "**Blank**" well respectively.

Positive Control: Prepare a well labeled as Positive Control.

Notes:

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

c. 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.

2. *p*NA Standard Curve: Add 5 µl of 0.1 M pNA Standard into 95 µl SK Assay Buffer to prepare 5 mM *p*NA Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 5 mM *p*NA Standard into each well individually. Adjust the volume to 100 µl/well with SK Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of *p*NA Standard.

3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. First, prepare working dilutions of each reagent including SK Substrate Buffer, SK Substrate and Plasminogen. Prepare SK Substrate Buffer working solution by diluting SK Substrate Buffer 1:1 with dH_2O. Prepare SK Substrate working solution by adding 11 µl of SK Substrate to 4 ul of dH_2O. Prepare Plasminogen working solution by adding 2 µl of stock Plasminogen solution to 18 µl of SK Assay Buffer.

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

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

Mix well. Add 60 µl Reaction Mix to **Positive Control, Reagent Background** and **Sample** wells. Add 60 µl Background Control Mix to the **Sample Background** well(s). Mix well. **Incubate the plate in a pre-heated plate reader for 5 min at 37 °C.** 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 and 60 µl Background Control Mix
Blank: 100 µl SK Assay Buffer

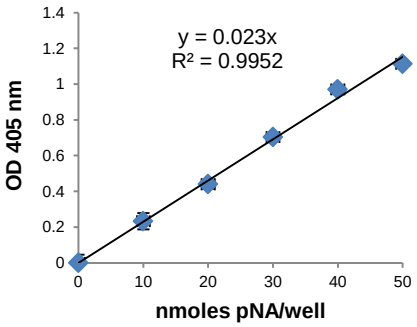
4. Measurement: Read the absorbance in a kinetic mode at OD 405 nm at 1 min intervals for 60 min at 37 °C. The *p*NA Standard Curve can be read in kinetic or endpoint mode (at the end of the incubation time).

5. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the *p*NA Standard Curve. If the Sample Background reading is significant, subtract the Sample Background reading from its paired Sample readings. Choose any two time points within the linear portion of the curve (t_1 & t_2) for each Sample. Apply the corrected Sample readings to the *p*NA Standard Curve to get B nmol of *p*NA generated during the reaction time ($\Delta t = t_2 - t_1$). Calculate the Sample Streptokinase Activity as shown below.

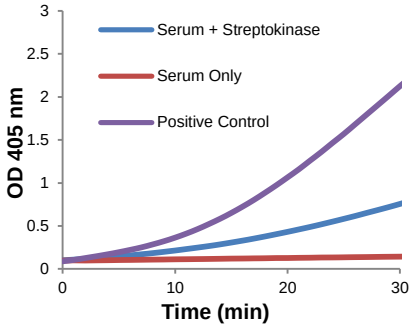
Sample Streptokinase Activity = B/(Δt*V) x D nmol/ml or **mU/ml**
Where B is the amount of *p*NA generated in the Sample well (nmol)
Δt (min)
V is the Sample volume added into the reaction well (ml)
D is the Sample dilution factor

Unit Definition: One Unit of Streptokinase is the amount of enzyme that generates 1 µmole of *p*NA per min at 37 °C.

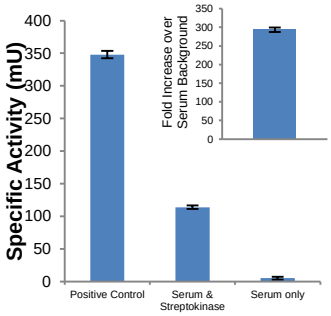
a)



b)



c)



Figures: a) *p*NA Standard Curve with 0-50 nmoles *p*NA/well. **b).** Positive control is Streptokinase and 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). **c).** Fold increase in absorbance with the addition of Streptokinase to serum-off-the-clot over Background (inset).

VIII. Related Products:

Tissue Plasminogen Activator (tPA) Activity Assay Kit (Colorimetric) (BV Cat# K178)
Plasmin Activity Assay Kit (Fluorometric) (BV Cat# K381)
Plasmin Inhibitor Screening Kit (Fluorometric) (BV Cat# K382)
Urokinase Inhibitor Screening Kit (Fluorometric) (BV Cat# K727)
Urokinase Activity Fluorometric Assay Kit (BV Cat# K728)
Plasmin Activity Assay Kit (Colorimetric) (BV Cat# K945)

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