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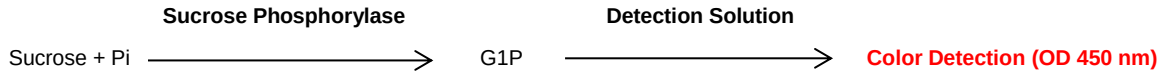
Sucrose Phosphorylase Activity Assay Kit (Colorimetric)

01/20

(Catalog # K2034-100; 100 assays, Store kit at -20°C)

I. Introduction:

Sucrose Phosphorylase (EC 2.4.1.7) is a transglucosidase enzyme, which plays a key role in the metabolism of sucrose, starch as well as other metabolites. It catalyzes the conversion of sucrose to α-D-glucose-1-phosphate (G1P) and D-fructose. It is present in both gram positive and negative bacteria such as in *Leuconostoc mesenteroides*, *Bifidobacterium longum*, *Pseudomonas sacclwrophila* and *Lactobacillus reuteri*. Sucrose Phosphorylase is used as a biocatalyst to produce fructose syrup in food industry and for determining inorganic phosphate in clinical analysis. **BioVision's Sucrose Phosphorylase Activity Assay Kit** can be used to determine the activity of Sucrose Phosphorylase in different microorganisms. The assay converts Sucrose to G1P, which is then detected by a set of enzymatic reactions to generate a colored product. The colored product can be measured by absorbance (OD 450 nm) and is directly proportional to the Sucrose Phosphorylase activity. BioVision's Sucrose Phosphorylase Activity Assay Kit is a rapid, sensitive and a convenient tool for measuring Sucrose Phosphorylase activity. It can detect as low as 10 mU under the assay conditions.



II. Applications:

- Measurement of Sucrose Phosphorylase activity in bacterial lysates
- Analysis of Sucrose Phosphorylase kinetics and inhibition

III. Sample Type:

- Bacterial lysates

IV. Kit Contents:

Components	K2034-100	Cap Code	Part Number
Sucrose Assay Buffer	25 ml	WM	K2034-100-1
Sucrose	1.2 ml	Orange	K2034-100-2
Sucrose Enzyme Mix	1 vial	Purple	K2034-100-3
Sucrose Substrate Mix	1 vial	Red	K2034-100-4
Sucrose Developer	1 vial	Green	K2034-100-5
G1P Standard	1 vial	Yellow	K2034-100-6
Sucrose Phosphorylase	1 vial	Blue	K2034-100-7

V. User Supplied Reagents and Equipment:

- Sonicator
- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- 50% glycerol
- Distilled water

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **Sucrose Assay Buffer and Sucrose:** Ready to use as supplied. Warm bottle to room temperature (RT) before use. Store at 4°C.
- **Sucrose Enzyme Mix and Sucrose Developer**: Reconstitute each vial with 220 µl of Sucrose Assay Buffer. Keep on ice while in use. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for two months at -20°C.
- **Sucrose Substrate Mix**: Reconstitute with 220 µl of water. Divide into aliquots and store at -20°C. Stable for two months at -20°C.
- **G1P Standard:** Reconstitute with 100 µl of water to generate 100 mM G1P Standard stock solution. Stable for two months at -20°C.
- **Sucrose Phosphorylase**: Add 100 µl of 50% glycerol (not included) to the vial. Vortex to mix and let it sit at RT for 5 min. Stable for two months at -20°C.

VII. Sucrose Phosphorylase Activity Assay Protocol:

1. **Sample Preparation:** Grow bacteria in 500 ml of any suitable growing medium (eg. LB or other chemically defined medium) at 37°C overnight. After incubation, harvest the cells by centrifuging at 10,000 x *g* for 20 min. Add 5 ml of ice-cold PBS per 1 gram of wet cell pellet. Sonicate the cells for 5 min at 4°C on ice and centrifuge at 10,000 x *g* for 15 min at 4°C. Transfer the clear supernatant to a new eppendorf tube. For each Sample type, add 2-10 µl of the supernatant into the desired well(s) of a clear, flat bottom 96-well plate labeled as Sample. Adjust the volume of each well to 50 µl using Sucrose Assay Buffer. **For Background Control well,** add 50 µl of Sucrose Assay buffer in separate well(s). **For Positive Control well,** add 5 µl of the reconstituted Sucrose Phosphorylase into the designated well(s). Adjust the volume to 50 µl/well using Sucrose Assay Buffer.
2. **Standard Curve Preparation:** Prepare 1 mM G1P Standard solution by adding 10 µl of the 100 mM G1P Standard stock solution to 990 µl of water. Add 0, 2, 4, 6, 8 and 10 µl of the 1 mM G1P Standard solution into the desired wells to generate 0, 2, 4, 6, 8 and 10 nmole/well of G1P Standard/well respectively. Adjust the volume of all wells to 50 µl/well using Sucrose Assay Buffer.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix and 50 µl of Background Control Mix as indicated in the table below:

	Reaction Mix	*Background Control Mix
Sucrose Assay Buffer	34 µl	44 µl
Sucrose	10 µl	--
Sucrose Enzyme Mix	2 µl	2 µl
Sucrose Substrate Mix	2 µl	2 µl
Sucrose Developer	2 µl	2 µl

Mix well. Add 50 µl of Reaction Mix to the wells containing Standard, Sample(s) and Positive Control and add 50 µl of Background Control mix to the Background Control well(s) respectively.

Note:

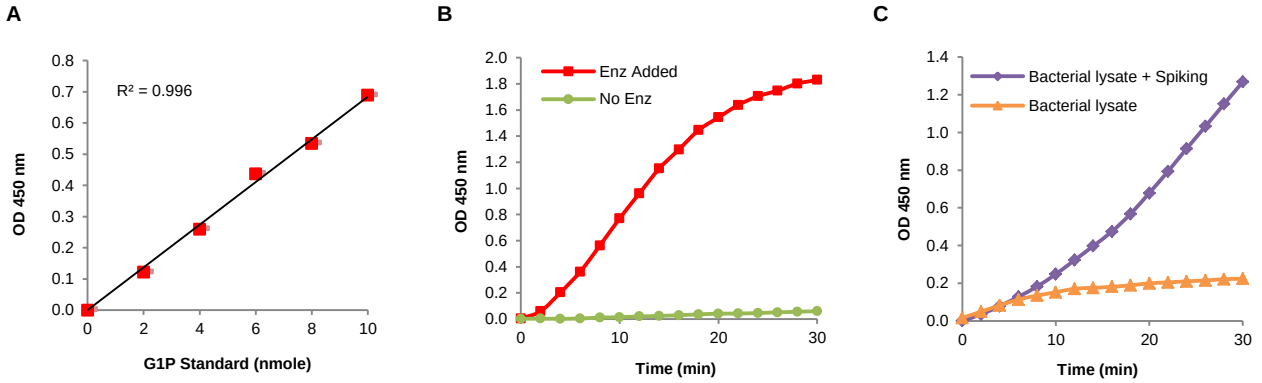
a) For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure that the readings are within the linear range of the Standard Curve.

4. **Measurement:** Measure the OD at 450 nm in kinetic mode at 25°C for 30 min.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings and the Background Control reading(s) from Sample readings respectively to get the corrected Sample readings. Plot the G1P Standard Curve. Choose any two time points within the linear portion of the curve (t_1 and t_2) for each Sample. Apply the corrected Sample readings to the G1P Standard Curve to get A nmol of G1P formed during the reaction time ($\Delta t = t_2 - t_1$). Calculate the Sucrose Phosphorylase activity of the Samples using the following equation:

Sample Sucrose Phosphorylase Activity = (A x D / Δt x M) ((nmol / min x µg)) = mU / µg

Where: **A** is the amount of G1P generated from the Standard Curve (nmol)
D is the Sample dilution factor (if applicable, D = 1 for Undiluted Samples)
Δt is the reaction time (in min)
M is the Sample added to the well (in µg)

Unit Definition: One unit is 1 µmole of G1P generated per min at pH 7 and 25°C.



Figures. A. G1P Standard Curve. **B.** Reaction curve of the Sucrose Phosphorylase activity. **C.** Sucrose Phosphorylase activity in bacterial lysates before and after spiking with 10 mU of the Sucrose Phosphorylase. Assay was performed according to the kit protocol.

VIII. Related Products:

Glycogen Phosphorylase Colorimetric Assay Kit (K179)
Fructose Assay Kit (Colorimetric) (K439)
Sucrose Colorimetric/Fluorometric Assay Kit (K626)
Maltose Phosphorylase Activity Kit (Fluorometric) (K353)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Glucose-1-Phosphate Colorimetric Assay Kit (K697)

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