ab284536 – Sucrose Phosphorylase Activity Assay Kit (Colorimetric)

For the measurement of Sucrose Phosphorylase activity in bacterial lysates.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284536

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, store the kit components as per the respective temperatures mentioned below. Use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Sucrose Assay Buffer	25 mL	-20°C
Sucrose	1.2 mL	-20°C
Sucrose Enzyme Mix	1 vial	-20°C
Sucrose Substrate Mix	1 vial	-20°C
Sucrose Developer	1 vial	-20°C
G1P Standard	1 vial	-20°C
Sucrose Phosphorylase	1 vial	-20°C

Materials Required, Not Supplied

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

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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.

Sucrose Phosphorylase Activity Assay Protocol

Sample Preparation:

1. Grow bacteria in 500 ml of any suitable growing medium (e.g., LB or other chemically defined medium) at 37°C overnight.
2. 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.
3. Transfer the clear supernatant to a new Eppendorf tube.
4. 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.
5. For Background Control well, add 50 µl of Sucrose Assay buffer in separate well(s).
6. 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.

Standard Curve Preparation:

1. Prepare 1 mM G1P Standard solution by adding 10 µl of the 100 mM G1P Standard stock solution to 990 µl of water.
2. 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.
3. Adjust the volume of all wells to 50 µl/well using Sucrose Assay Buffer.

Reaction Mix Preparation:

1. 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

2. 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.
   Δ Note: *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.*

Measurement

1. Measure the OD at 450 nm in kinetic mode at 25°C for 30 minutes.

Calculation:

1. For 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.
2. Plot the G1P Standard Curve.
3. Choose any two time points within the linear portion of the curve (t1 and t2) for each Sample.
4. Apply the corrected Sample readings to the G1P Standard Curve to get A nmol of G1P formed during the reaction time ($\Delta t = t2-t1$).
5. 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 = amount of G1P generated from the Standard Curve (nmol)
D = Sample dilution factor (if applicable, D = 1 for Undiluted Samples)
Δt = Reaction time (min)
M = Sample added to the well (µg)

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

Technical Support

For all technical or commercial enquiries please go to:

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