ab65334 – Glucose and Sucrose Assay Kit

For measurement of glucose and sucrose levels from various biological samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab65334

Storage and Stability

The entire ELISA kit may be stored at -20°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 2	25 mL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Invertase	1 vial	-20°C
Developer Mix B	1 vial	-20°C
Sucrose Standard	100 µL	-20°C

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Glucose Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Glucose Probe (in DMSO). Developer Mix B was previously labelled as Development Enzyme Mix II and Glucose Enzyme Mix (Lyophilized). The composition has not changed.

Reagent Preparation

OxiRed™ Probe: Ready to use as supplied. Warm to room temperature prior to use to melt frozen DMSO. Store at –20°C, protect from light and moisture. Use within two months.

Invertase, Developer Mix B: Dissolve in 220 µl Assay Buffer 2. Aliquot and store at –20°C. Use within two months.

Assay Protocol:

1. Standard Curve Preparations:

For colorimetric assay, dilute the Sucrose Standard to 1 nmol/µl by adding 10 µl of the Sucrose Standard to 990 µl of Assay Buffer 2, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer 2 to generate 0, 2, 4, 6, 8, 10 nmol/well of Sucrose Standard.
For fluorometric assay, dilute the Sucrose Standard solution to 0.1 nmol/µl by adding 10 µl of the Sucrose Standard to 990 µl of Assay Buffer 2, mix well. Then take 20 µl into 180 µl of Assay Buffer 2. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer 2 to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Sucrose Standard. Fluorometric assay is 10-100 fold more sensitive than colorimetric assay.

2. Sample Preparations:

Prepare test samples in 50 µl/well with Assay Buffer 2 in a 96-well plate. Serum can be directly diluted in the Assay Buffer 2. We suggest testing several doses of your sample to make sure the readings are within the standard curve linear range. For Sucrose detection, prepare two wells for each sample. To one well, add 2 µl of Invertase to convert sucrose to glucose by incubating the invertase reaction at 37°C for 30 min before adding Glucose Assay Mix in next step. To the other vial, omit Invertase the assay detects free glucose only. Sucrose = Total Glucose – Free Glucose.

Δ Note: *2 µl of invertase must be added to each well of the Sucrose Standard to convert sucrose standard to glucose for either glucose or sucrose assay.*

3. **Glucose Assay Mix:** Mix enough reagent for the total number of assays (samples + standards) to be performed.
   For each well, prepare a total 50 µl Reaction Mix containing:
   46 µl Assay Buffer 2
   2 µl OxiRed™ Probe
   2 µl Developer Mix B
4. Mix well. Add 50 µl of the Reaction Mix to each well containing the Sucrose Standard or test samples. Mix well. Incubate the reaction for 30 minutes at 37°C, protect from light.
5. Measure OD 570nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.
6. Correct background by subtracting the value derived from the 0 sucrose control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Glucose concentrations of the test samples can then be calculated based on the standard curve you generated, the dilution factor, and volume of your samples added into the wells. Sucrose = Total Glucose – Free Glucose.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus