



Rev 03/19

# **Glucose Isomerase Activity Assay Kit (Colorimetric)**

(Catalog # K491-100; 100 assays; Store at -20°C)

# I. Introduction:

Glucose (Xylose) Isomerase (GI) (EC 5.3.1.5) can catalyze the reversible conversion of glucose to fructose, and xylose to xylulose. GI has important industrial applications in the production of high-fructose corn syrup (HFCS). It is known HFCS is sweeter than glucose; thus it is widely used in foodstuff and pharmaceutical products. Additionally, additional research on using Glucose Isomerase in the production of biofuels has increased. BioVision's Glucose Isomerase Activity Assay kit provides a quick and easy method for monitoring GI activity in various samples. In this Assay, Glucose Isomerase converts Fructose into Glucose. This isomerization is detected through a series of enzymatic reactions generating an intermediate that reacts with the Probe producing a stable chromophore with strong absorbance at OD = 570 nm. The assay is simple, sensitive and can detect Glucose Isomerase Activity as little as 50  $\mu$ U in a variety of samples.



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# II. Application:

Measurement of Glucose Isomerase Activity.

### III. Sample Type:

- Purified protein/preparations;
- Bacterial Lysates: E. coli, Streptomyces murinus, etc.

#### IV. Kit Contents:

Components	K491-100	Cap Code	Part Number
GI Assay Buffer	25 ml	WM	K491-100-1
GI Probe (in DMSO)	200 µl	Red	K491-100-2
GI Substrate	200 µl	Orange	K491-100-3
GI Converter	1 vial	Purple	K491-100-4
GI Developer	1 vial	Green	K491-100-5
Glucose Standard (100 mM)	100 µl	Yellow	K491-100-6
GI Positive Control (in Ammonium Sulfate)	50 µl	Blue	K491-100-7

#### V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (96-well plate reader)
- Ammonium Sulfate Solution (Saturated, 4.1 M), BioVision Cat# 7096
- Dounce homogenizer (Cat# 1998)

### VI. Storage Conditions and Reagent Preparation:

- Store kit at -20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.
- GI Converter and GI Developer: Reconstitute each vial with 220 µl GI Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- GI Probe: Ready to use as supplied. Warm to room temperature prior to use. Store at -20°C, protect from light and moisture. Use within two months.
- GI Positive Control: Note GI is extremely unstable when not in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> Solution. Mix GI Positive Control well and take a 10 µl GI aliquot; centrifuge aliquot at 10,000 x g for 5 min at RT. Carefully remove the supernatant and dissolve pellet with 100 µl Assay Buffer. Mix thoroughly. Use this solution within 4 hours. Store rest of GI Positive Control in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution at 4°C. Use within two months.

#### VII. Glucose Isomerase Assay Protocol:

- 1. Sample Preparation: For bacterial lysate: grow bacteria at desired conditions, collect culture and spin down at 10,000 x g, 20 min at 4°C. Remove the supernatant and homogenize pellets (100 mg) with 1 ml ice-cold GI Assay Buffer using homogenizer for 2 min on ice, and place on ice for 10 minutes. Centrifuge at 10,000 x g for 10 min at 4°C. Collect the supernatant. Use ammonium sulfate precipitation to remove interferences.: Aliquot tissue samples (100 µl) to clean centrifuge tubes, add saturated 4.32 M ammonium sulfate (BioVision Cat. # 7096) reaching 65% saturation (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate). Place samples on ice for 30 min. Spin down samples at 10,000x g at 4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume with GI Assay Buffer. For each sample to be tested add in two parallel wells the same volume (2-50 µl) into a 96 well clear plate designated as Sample [S] and Sample Background Control [BC]. Positive Control: add 2-20 µl of GI Positive Control. Adjust final volume of all samples and positive control(s) to 50 µl with GI assay buffer.
  - a. For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.
  - b. To control for sample background, prepare parallel sample wells as sample background controls.
  - c. If you would like to determine specific GI activity in the samples use BioVision's BCA Protein Assay Kit Reducing Agent Compatible (K818-1000) or similar to determine protein concentration in samples.





- 2. Glucose Standard Curve: Dilute the Glucose Standard to 1 nmol/μl (1 mM) by adding 10 μl of the Glucose Standard to 990 μl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8 and 10 μl of 1 mM Glucose Standard into a series of wells in a 96 clear well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of Glucose Standard. Adjust volume to 50 μl/well with Assay Buffer.
- 3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix
GI Assay Buffer	42 µl	44 µl
GI Converter	2 µl	2 µl
GI Developer	2 µl	2 µl
OxiRed <sup>™</sup> Probe	2 µl	2 µl
GI Substrate	2 µl	

Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and Samples [S] and 50 µl of Background Control mix to each well containing the Sample Background Controls [BC]. Mix well.

4. Measurement: Measure absorbance immediately at OD = 570 nm in kinetic mode for 5-60 min at 37°C.

**Note:** Incubation time depends on the Glucose Isomerase activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points ( $t_1 \& t_2$ ) in the linear range to calculate the GI activity of the samples.

5. Calculation: Subtract the 0 standard reading from all standard readings. Plot the Glucose standard curve. Correct sample reading by subtracting the value derived from the sample background control from all sample readings. Calculate the GI activity of the test sample:  $\Delta OD = A_2 - A_1$ . Apply the  $\Delta OD$  to the Glucose standard curve to get B nmol of Glucose generated by Glucose Isomerase during the reaction time ( $\Delta t = t_2 - t_1$ ).

# Sample Glucose Isomerase Activity = B/(∆t X V) x Dilution Factor = nmol/min/ml/ = mU/mI

Where:

 $\mathbf{B}$  = the Glucose amount from standard curve (nmol).  $\Delta \mathbf{t}$  = the reaction time (min).  $\mathbf{V}$  = the sample volume added into the reaction well (ml).

Unit Definition: One unit of Glucose Isomerase is the amount of enzyme that isomerizes 1.0 µmol of glucose per min at pH 8.0 at 37°C.



Figure: (a) Glucose standard curve. (b) Kinetic measurement of Glucose Isomerase activity from Streptomyces Lysates (µg protein): Sample A (26 µg), Sample B (52 µg). (C) Relative GI Activity was calculated in lysates prepared from *E. coli.* and *Streptomyces*. Assays were performed following kit protocol.

# VIII. RELATED PRODUCTS:

Glucose and Sucrose Assay Kit (K616) Glucose Uptake Colorimetric Assay Kit (K676) Glucose Uptake Fluorometric Assay Kit (K666) NAD/NADH Quantification Kit (K337) PicoProbe<sup>™</sup> Glucose-6-Phosphate Assay Kit (K687) Phosphoglucomutase Assay Kit (K774) Glucose-6-Phosphate Colorimetric Assay Kit (K657) Glucose-6-Phosphate Dehydrogenase Assay Kit (K757) Fructose Assay kit (K619) Hexokinase Assay Kit (K789) Glucose Dehydrogenase Activity Assay Kit (K679) Free Glycerol Colorimetric Assay Kit (K634)

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