

Glucose Isomerase

HAMPTON
RESEARCH

Solutions for Crystal Growth

User Guide

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Overview

Glucose Isomerase is supplied as a crystal suspension in the following medium:

- 33 mg/ml crystalline Glucose Isomerase
- 6 mM TRIS hydrochloride pH 7.0
- 120 mg/ml Ammonium sulfate (0.91 M)
- 1 mM Magnesium sulfate
- Dry substance: A 280 nm ratio for Glucose Isomerase
0.96 mg/ml: A 280 nm - 1.00

The crystals in the supplied suspension are mostly 50-150 micron. In addition to crystals, there are fragments of crystals produced in the bulk handling and bottling. The suspension does not contain preservative.

Source

Purified from *Streptomyces rubiginosus*.

Crystallized suspension is prepared via 5 times batch crystallization from the cell extract of *Streptomyces rubiginosus*.

Storage

Store at 2°C to 5°C. Do not freeze.

Preparing for Crystallization

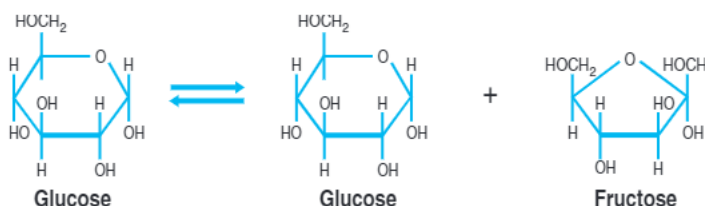
Before removing an aliquot or the entire contents of Glucose Isomerase, gently mix the solution of crystalline Glucose Isomerase to create a homogeneous crystalline suspension. Dialyze the suspension versus 10 mM HEPES pH 7.0 (or other buffer which does not bind Magnesium), 1 mM Magnesium chloride. It is recommended the dialysis buffer be changed several times to remove the TRIS hydrochloride and Ammonium sulfate. Filter the dialyzed protein through a 0.2 micron filter and store at 2°C to 5°C. Do not freeze. The supplied crystalline Glucose Isomerase is stable for years. A sterile filtered solution (non-crystalline) of Glucose Isomerase in 10 mM Tris pH 7.0, 1 mM Magnesium chloride stored in a sterile container at 2°C to 5°C is stable for years. Avoid microbial contamination. Glucose Isomerase is rapidly denatured below pH 5.0.

Specifications

Glucose Isomerase molecular weight = 173,000 daltons

Glucose Isomerase is a tetramer composed of four identical polypeptides of 43,000 daltons. On a SDS-PAGE the enzyme appears as a single band at 43,000 and on a Native Gel Electrophoresis the enzyme runs near 160,000 to 170,000.

Glucose Isomerase catalyzes the isomerization reaction shown below:



The reaction is reversible and goes to equilibrium, approximately equal concentrations of fructose and glucose: The enzyme is more active with xylose, thus the original scientific name is D-xylose ketol-isomerase EC 5.3.1.5.

Enzymatic Activity

Although this does not fulfill the traditional criteria of enzyme activity assays, the conversion of glucose to fructose as the measure of activity has been vastly used in literature.

Industry used unit: 1 GIU is 1 micromole glucose converted to fructose in one minute at pH 7, temperature 60°C, 300 g/l glucose concentration.

In the industrial assay one milligram of this GI has an activity of 29 GIU. More convenient in general laboratory practice is to use pure fructose as the substrate and measure the production of glucose by any of the numerous glucose assay methods. Use lower temperature, like 30°C. The reaction runs equally well in the direction fructose to glucose.

Solubility and Crystallization

Glucose Isomerase is most stable between pH 6.0 and 8.0, and crystallizes well in this pH range. Glucose Isomerase is rapidly denatured below pH 5.0.

Ammonium sulfate is an appropriate crystallization reagent for Glucose Isomerase. Crystals are also produced with magnesium and calcium salts, as well as sulfates, chlorides, and nitrates. Suggested crystallization conditions (vapor diffusion or batch, 20 - 30 mg/ml protein concentration):

- 1.5 - 2.5 M Ammonium sulfate pH 6.0 - 9.0
- 10 - 15% w/v PEG 4,000-8,000, 0.2 M salt *, 6.0 - 9.0
- 0.6 - 1.0 M Sodium citrate tribasic dihydrate, pH 6.0 - 8.0
- 0.1 - 0.3 M Magnesium formate dihydrate
- 1.0 M Sodium formate, pH 5.0 - 7.0
- 10 - 20% v/v MPD, 0.2 M salt *, pH 6.0 - 9.0
- 10 - 20% v/v PEG 400, 0.2 M salt *, pH 6.0 - 9.0
- 10 - 20% 2-Propanol, 0.2 M salt *, pH 6.0 - 9.0

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* = 0.2 M salt such as Ammonium sulfate, Magnesium chloride, Magnesium acetate, Calcium acetate, or Ammonium acetate. Experimentation with other "secondary salts" has also been successful in crystallizing Glucose Isomerase.

Cobalt and Magnesium stabilize Glucose Isomerase. Calcium inhibits the enzymatic activity and stabilizes Glucose Isomerase.

The isoelectric point of Glucose Isomerase is approximately 3. The enzyme does not behave well during isoelectric focusing since the protein is denatured near the isoelectric pH.

Recommended protein concentration for crystallization is 10 to 30 milligrams per milliliter.

Temperature Information

Glucose Isomerase responds well to temperature crystallization methods and is less soluble at cold temperatures than warm temperatures. Proteins can be divided into 3 classes of temperature dependent solubility.

- Class 1: Solubility increases exponentially by increasing temperature. (Most common).
- Class 2: Very little or no temperature effect.
- Class 3: Solubility decreases when temperature increases. Glucose Isomerase falls into Class 1.

The supplied crystalline suspension of Glucose Isomerase can be dissolved by heating the solution to 55 to 60°C and recrystallized by slow cooling. Crystal size is dependent upon cooling rate and mechanical variables such as vibration or stirring.

Molar Extinction Coefficient

The calculated molar extinction coefficient for glucose isomerase is 45660 M⁻¹cm⁻¹.

Reference: Glucose isomerase from *Streptomyces rubiginosus* – potential molecular weight standard for small angle X-ray scattering. Kozak, M., J. Appl. Cryst. (2005). 38, 555–558.

References

Carrell, H. L. et al., Proc. Natl. Acad. Sci. USA, Vol 86, pp 4440-4444, June 1989.

Technical Support

Inquiries regarding Glucose Isomerase, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

Related Products

HR7-100	Glucose Isomerase	1 gram
HR7-102	Glucose Isomerase	100 milligrams

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