CREATIVE BIOMART®
Assay Kit

Glycogen Branching Enzyme Assay Kit

Product Information

Cat.No. Kit-3430

Product Overview

The Glycogen Branching Enzyme Assay Kit is used for determining Glycogen Branching Enzyme activity in various samples. Glycogen branching enzyme activity is determined by iodine assay. And it can be measured at 660 nm. The Glycogen Branching Enzyme activity in the sample is then determined by comparing the O.D. of samples to the standard.

Storage

2-8°C

Kit Components

Microplate, 1 X 96-well plate, 4°C
Standard (5 µmol), 1 vial (Lyophilized), 4°C
Assay Buffer, 4 X 30 ml (ready to use), 4°C
Reaction Buffer 1, 6 ml (ready to use), 4°C
Reaction Buffer 2, 8 ml (ready to use), 4°C
Substrate, 1 vial (Lyophilized), 4°C
Reaction Dye, 1 ml (ready to use), 4°C

Materials Required but Not Supplied

Microplate reader capable of measuring absorbance at 660 nm
Pipettes and pipette tips
Deionized or distilled water
Convection oven (37°C)

Technical Notes

Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
Store the kit at 4°C at all times.
Reaction Dye should be store at 4°C and protect from light.

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Briefly spin down the reagents before use.
It is highly recommended that the standards and samples be assayed in at least duplicates.
Change pipette tips between the addition of different reagent or samples

Preparation

Sample Preparation (for liquid samples):
Add 0.1 ml of liquid samples into 0.9 ml of Assay buffer in a microcentrifuge tube, mix well. Centrifuge the tube 10000 X g at 4 °C for 10 minutes, collect the supernatant into a new tube and keep it on ice before assay. Undiluted samples can be aliquoted and stored at -20°C or belowfor up to 1 month. Avoid repeated freeze-thaw cycles.
Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Centrifuge samples 10000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.
REAGENT PREPARATION □
Substrate: Reconstitute the Substrate with 1 ml of distilled water. Allow the Substrate to heat for 1 minute at boiling water bath or heat plate. Make sure the Substrate is dissolved completely before use. The reconstituted Substrate can be stored at 4°C for up to 1 week. □
Standard: Reconstitute the Standard with 1 ml of distilled water to yield a stock concentration of 5 µmol/ml (5 mmol/L). Allow the Standard to sit for few minutes with gentle agitation to make sure the Standard is dissolved completely before use. The reconstituted standard stock can be aliquoted and stored at -20°C for up to 1 month. Dilute 400 µl of the reconstituted Standard stock with 600 µl distilled water before use. The concentration of the working standard solution will be 2 µmol/ml (2 mmol/L).

Assay Protocol

All materials should be equilibrated to room temperature (RT) before use. Standards and samples should be assayed in at least duplicates.
1. Add 40 µl per (diluted) samples into each microcentrifuge tube.
2. Add 40 µl of distilled water into control tube.
3. Add 10 µl of distilled water into standard tube.

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4. Add 50 µl of distilled water into blank tube.
5. Add 60 µl of Reaction Buffer 1 per tube into each Sample, Control, Standard and Blank tube.
6. Add 10 µl of reconstituted Substrate into each Sample and Control tube.
7. Mix well and closed lid of the tube with cap lock. Incubate all tubes at 37°C for 20 min.
8. Then incubate all tubes at boiling water bath for 1 min.
9. Transfer all reagent into the microplate.
10. Add 80 µl of Reaction Buffer 2 per well into each Sample, Control, Standard and Blank tube.
11. Add 40 µl of Standard into standard well.
12. Add 10 µl of Reaction Dye per well into all wells.
13. Gently tap the plate to ensure thorough mixing.
14. Incubate the plate at RT for 10 min.
15. Read the OD with a microplate reader at 660 nm immediately.

Summary of Glycogen Branching Enzyme Assay Procedure

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	-	-	-
Distilled water	-	40 µl	10 µl	50 µl
Reaction Buffer 1	60 µl	60 µl	60 µl	60 µl
Substrate	10 µl	10 µl	-	-
Mix well and incubate all tubes at 37°C for 20 min.				
Incubate all tubes at boiling water bath for 1 min				
Reaction Buffer 2	50 µl	50 µl	50 µl	50 µl
Standard	-	-	40 µl	-
Reaction Dye	10 µl	10 µl	10 µl	10 µl
Mix thoroughly, incubate the tubes at RT for 10 min.				
Read the OD with a microplate reader at 660 nm immediately.				

Analysis

1. Unit Definition: One unit of Glycogen Branching Enzyme activity is defined as the enzyme decompose 1 µmol soluble starch per minute.
2. Calculate the average absorbance values for each set of samples, standards, controls and blank.

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3. Calculation:
A. Definition:
CProtein: the protein concentration, mg/ml;
CStandard: the standard concentration, 2 μmol/ml;
W: the weight of sample, g;
VStandard: the volume of the standard, 40 μl = 0.04 ml;
VSample: the volume of reaction sample, 40 μl = 0.04 ml;
Vtotal: the total volume of sample in Assay buffer, 1 ml;
N: sample dilution factor = 10;
T: the reaction time, 20 minutes.
B. Formula: a). According to the protein concentration of sample
Glycogen Branching Enzyme activity (U/mg) = [(CStandard × VStandard) × (ODControl - ODSample)] / [(ODStandard- ODBlank) X (VSample × CProtein) X T] = 0.1 × (ODControl - ODSample) / [(ODStandard - ODBlank) X CProtein]
b). According to the weight of sample
Glycogen Branching Enzyme activity (U/g) = [(CStandard × VStandard) × (ODControl - ODSample)] / [(ODStandard- ODBlank) X (W × VSample / Vtotal) X T] = 0.1 × (ODControl - ODSample) / [(ODStandard - ODBlank) X W]
c). According to the volume of sample
Glycogen Branching Enzyme activity (U/ml) = N X [(CStandard × VStandard) × (ODControl - ODSample)] / [(ODStandard- ODBlank) X VSample X T] = 10 X 0.08 X (ODSample- ODBlank) / [(ODStandard- ODBlank) X 0.04 X 20] =1 X (ODSample - ODBlank) / (ODStandard - ODBlank)

Sensitivity
The detection range is from 0.01 mmol/L - 2 mmol/L

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