CREATIVE BIOMART®
Assay Kit

UDP-Galactosyltransferase Assay Kit

Product Information

Cat.No. Kit-3311

Product Overview

The kit assay is based on the transfer of radioactively labeled(*) galactose from UDP-galactose to N-acetylD-glucosamine by UDP-galactosyltransferase. The reaction product, a radiolabeled N-acetylD-lactosamine, is then purified by ion-exchange chromatography and quantified.

Description

UDP-galactosyltransferase (b4GalT1) is a trans-Golgi membrane bound protein that participates in protein processing in the Golgi apparatus. UDP-galactosyltransferase catalyzes the transfer of galactose from UDP-galactose to N-acetyl-D-glucosamine. The exclusive location of UDP-galactosyltransferase in the Golgi apparatus in the vast majority of tissues and cells, makes it a convenient biochemical marker for Golgi membranes.

Applications

The kit provides a convenient method for quantitative estimation of the UDP-galactosyltransferase activitity in cell or tissue extracts.

Storage

The kit is shipped on dry ice. The Mini-Columns can be stored at room temperature. The UDP-Gal Solution and Acceptor Solution should be stored at –20 °C. All remaining components can be stored at 2-8 °C.

Size

100 tests

Kit Components

Assay Buffer 500 ul
Manganese Chloride Solution 500 ul
Acceptor Solution 500 ul
UDP-Gal Solution 500 ul

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Cleanup Resin 60% suspension 50 ml
Mini-Columns 100 each
Elution Solution 100 ml

Materials Required but Not Supplied

Microcentrifuge tubes
Scintillation vials
Liquid Scintillation Cocktail, Ultima Goldä LSC Cocktail
Scintillation counter
37 °C water bath or a heating plate
Microcentrifuge
Radiolabeled Uridine 5¢-diphosphogalactose (3H or 14C label in the galactose moiety)

Technical Notes

Prior to use, thaw the kit components and mix until homogeneous.

Assay Protocol

Use ultrapure water (17 MW×cm or equivalent) throughout the procedure. Place water (100 ml for each sample) on ice for use in step 5.
It is highly recommended to perform the assays in duplicate.
1. Determine the protein content of the test samples. It is recommended to use 20–50 ug of total protein per assay.
Note: Since the method utilizes a cleanup ion-exchange column, it is important to keep the salt concentration in all the samples approximately equal. The salt concentration of the buffer should be ≤100 mM.
2. For each test prepare two sets of reaction mixtures in separate microcentrifuge tubes (see Table 1): one with Acceptor Solution (test) one without Acceptor Solution (background control)
Table 1. Reaction Mixtures

	Test (with Acceptor)	Background Control (without Acceptor)
Assay Buffer	5 ul	5 ul
Manganese Chloride Solution	5 ul	5 ul
UDP-Gal Solution (10 mM)	5 ul	5 ul

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Acceptor Solution 5 ul –
Radiolabeled UDP-Gal x ul (0.1–1 uCi) x ul (0.1–1 uCi)
Sample 20–50 ug 20–50 ug
Water Adjustable – bring final volume of Reaction Mixture to 50 ul
3. Vortex the tubes briefly and incubate at 37 °C for 1 hour.
4. During the incubation, prepare the cleanup columns. Mix the Cleanup Resin until homogenous and load 0.5 ml into each Mini-Column. Allow the fluid to pass through the column. Place the column in a 2 ml microcentrifuge tube and spin very briefly to remove remaining liquid. Transfer the Mini-Column to a fresh 2 ml microcentrifuge tube.
5. At the end of the 1 hour incubation, stop the reaction by the addition of 100 ul of ice-cold water to each tube.
6. Load the reaction mixture onto the cleanup column and let the solution absorb into the resin. Add 0.4 ml of the Elution Solution. Let the liquid pass through.
7. Using the same microcentrifuge tube, add an additional 0.4 ml of the Elution Solution. Let the liquid pass through and spin the column briefly.
8. Keep the combined eluate from steps 6–7 and discard the columns. Mix 0.5 ml of the eluate with scintillation liquid and count in a liquid scintillation counter.
9. Determine the radioactivity (Total cpm) of the radioactive substrate by diluting 0.1–1 uCi of the radioactively labeled UDP-Gal (same volume, x ml, used in a single assay) with 0.5 ml of water. Mix with scintillation liquid and count in a liquid scintillation counter.

Analysis

1. Calculate the specific radioactivity (cpm/nmol): Divide the Total cpm (step 9) by 50 (each assay uses 50 nmol of UDP-Gal).
2. Calculate the enzymatic specific activity of the sample according to the following equation:
Specific Activity = [(cpm test – cpm background) x 1.9]/(Spec Rad x protein x time)
Specific Activity (mU/mg protein) – specific activity of the sample in milliunits per mg protein
Spec Rad (specific radioactivity, Calculation 1) – radioactivity per nmol of UDP-Gal (cpm/nmol)
1.9 – Sample volume correction factor. This factor is required since only 0.5 ml out of 0.95 ml eluate was sampled for cpm counting.
Protein – amount of protein in the assay (mg)

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Time – the duration of the reaction (minutes)
Unit definition: one unit of enzyme transfers 1 mmole of galactose from UDP-galactose to N-acetylD-glucosamine per minute at pH 7 at 37 °C.

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