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Product Information

UDP-Galactosyltransferase Assay Kit

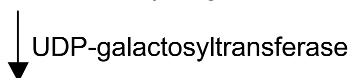
Catalog Number **CS1050**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
UDP-galactosyltransferase (β4GalT1) 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.

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

UDP- Galactose* + N-acetyl-D-glucosamine



UDP + N-acetyl-D-lactosamine*

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

Components
The kit is sufficient for 100 tests

Component	Amount
Assay Buffer Catalog Number A9230	500 μl
Manganese Chloride Solution Catalog Number M3945	500 μl
Acceptor Solution Catalog Number A9355	500 μl
UDP-Gal Solution Catalog Number U6633	500 μl
Cleanup Resin 60% suspension Catalog Number C0243	50 ml
Mini-Columns Catalog Number C2728	100 each
Elution Solution Catalog Number E5281	100 ml

Reagents and Equipment Required but Not Provided
- Microcentrifuge tubes (Catalog Number T2795 or equivalent)
- Scintillation vials (Catalog Number Z376825)
- Liquid Scintillation Cocktail, Ultima Gold™ LSC Cocktail (Catalog Number L8286 or equivalent)
- Scintillation counter
- 37 °C water bath or a heating plate
- Microcentrifuge
- Radiolabeled Uridine 5′-diphosphogalactose (^{3}H or ^{14}C label in the galactose moiety)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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

Storage/Stability
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.

Procedure

Use ultrapure water (17 MΩ·cm or equivalent) throughout the procedure. Place water (100 μl 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 μg 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 μl	5 μl
Manganese Chloride Solution	5 μl	5 μl
UDP-Gal Solution (10 mM)	5 μl	5 μl
Acceptor Solution	5 μl	–
Radiolabeled UDP-Gal	x μl (0.1–1 μCi)	x μl (0.1–1 μCi)
Sample	20–50 μg	20–50 μg
Water	Adjustable – bring final volume of Reaction Mixture to 50 μl	

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 (Catalog Number C0243) until homogenous and load 0.5 ml into each Mini-Column (Catalog Number C2728). 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 μl 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 μCi of the radioactively labeled UDP-Gal (same volume, x μl, used in a single assay) with 0.5 ml of water. Mix with scintillation liquid and count in a liquid scintillation counter.

Calculations

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:

$$\text{Specific Activity} = \frac{(\text{cpm}_{\text{test}} - \text{cpm}_{\text{background}}) \times 1.9}{\text{Spec Rad} \times \text{protein} \times \text{time}}$$

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)

Time – the duration of the reaction (minutes)

Unit definition: one unit of enzyme transfers 1 μmole of galactose from UDP-galactose to N-acetyl-D-glucosamine per minute at pH 7 at 37 °C.

References

1. Berger, E.G., Ectopic localizations of Golgi glycosyltransferases. *Glycobiology*, **12**, 29R-36R (2002).
2. Bella, A., Jr., *et al.*, Human plasma uridine diphosphate galactose-glycoprotein galactosyl-transfertase. Purification, properties and kinetics of the enzyme-catalysed reaction. *Biochem. J.*, **167**, 621-628 (1977).
3. Bretz, R., and Staubli, W., Detergent influence on rat liver galactosyltransferase activities towards different acceptors. *Eur. J. Biochem.*, **77**, 181-192 (1977).

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Troubleshooting Guide

Problem	Possible Cause	Solution
The background signal is too high. **or** Large differences between duplicates	Contamination of the scintillation counter or vials with other isotopes	Count the vials without the sample.
	High salt concentration in the samples	Dilute the samples with water or low ionic-strength buffer to reduce salt concentration in the samples to ≤100 mM.
Sample signal is lower than expected.	Reaction temperature is lower than 37 °C.	Check/change the incubation device.
	Samples are too dilute.	Use 20 µg or more protein per assay. Extend the incubation time.

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