# Universal Phosphatase-Coupled Glycosyltransferase Assay

Zhengliang L Wu, Cheryl M Ethen, Brittany Prather, Miranda Machacek, and Weiping Jiang | R&D Systems, Inc., 614 McKinley Pl. NE, Minneapolis, MN, 55413


## ABSTRACT

The non-radioactive phosphatase-coupled glycosyltransferase assay described here offers a new method for measuring glycosyltransferase activity. This assay couples specific phosphatases with glycosyltransferase reactions to quantitatively release inorganic phosphate from the leaving nucleoside phosphates produced during the glycosyltransferase reactions. The released phosphate group is then detected using colorimetric malachite-based reagents. Because the amount of phosphate released is directly proportional to the sugar molecule transferred in a glycosyltransferase reaction, this method can be used to obtain accurate kinetic parameters of the glycosyltransferase. The assay can be performed in multi-well plates and quantitated by a plate reader, making it amenable to high-throughput screening. It has been successfully applied to all glycosyltransferases available to us, including glucosyltransferases, N-acetylglucosaminyltransferases, galactosyltransferases, N-acetylgalactosyltransferases, fucosyltransferases, and sialyltransferases. The method is demonstrated using *Clostridium difficile* toxin B (a protein O-glucosyltransferase), human KTELC1 (a glucosyltransferase homolog to Rumi from *Drosophila*) and human sialyltransferase ST6GAL1.

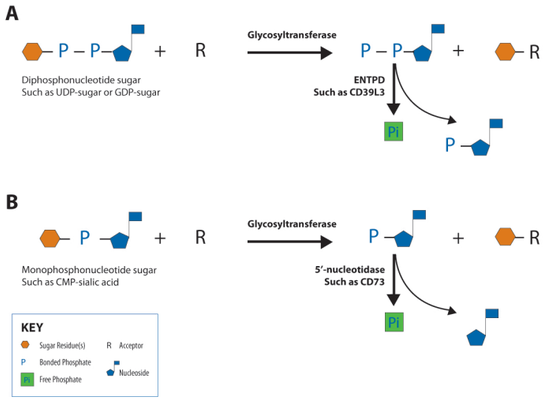

## INTRODUCTION

- In contrast to DNA and proteins that are synthesized based on templates, all glycans are synthesized by glycosyltransferases. Currently, there are more than 90 known families of glycosyltransferases. The majority of these enzymes are classified as Leloir enzymes. These enzymes use nucleotide sugars as donor substrates and generate leaving groups of nucleoside phosphate. Traditionally, glycosyltransferases are assayed by monitoring the transfer of radiolabeled sugars from donor to acceptor molecules. The detection of radioactive transfer requires the separation of products from substrates, which is typically achieved using methods such as column or thin layer chromatography. Various non-radioactive methods have been developed for glycosyltransferase assays as well. However, none of these methods can be regarded as universal.
- Here, we present a universal non-radioactive glycosyltransferase assay.

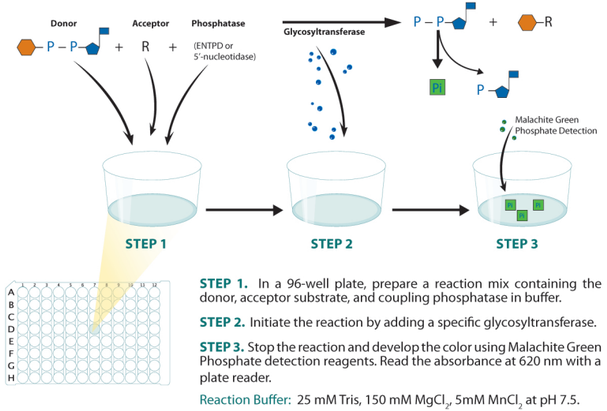


## METHODS

### PHOSPHATASE-COUPLED GLYCOSYLTRANSFERASE REACTIONS




### ASSAY PROCEDURE




**STEP 1.** In a 96-well plate, prepare a reaction mix containing the donor, acceptor substrate, and coupling phosphatase in buffer.

**STEP 2.** Initiate the reaction by adding a specific glycosyltransferase.

**STEP 3.** Stop the reaction and develop the color using Malachite Green Phosphate detection reagents. Read the absorbance at 620 nm with a plate reader.

Reaction Buffer: 25 mM Tris, 150 mM $MgCl_2$, 5mM $MnCl_2$ at pH 7.5.

### FIGURE 1

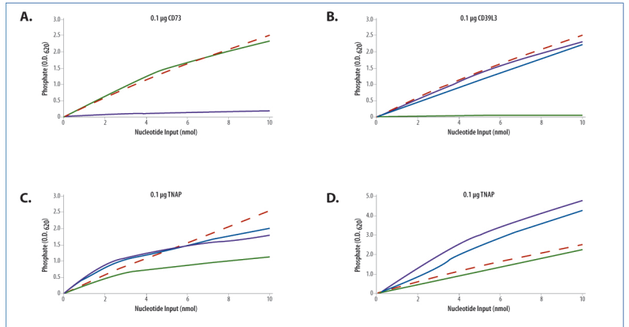



**FIGURE 1 Common leaving nucleotides can be dephosphorylated by specific phosphatases.** CMP (green), GDP (purple) and UDP (blue), were treated with CD73 **(A)**, CD39L3 **(B)** and TNAP **(C, D)**. Reactions with phosphate content higher than 4 nmol were diluted before measurement and absorbances were obtained by multiplying the observed O.D.s by the dilution factors. Dashed red lines represent phosphate standard curves.

### FIGURE 2

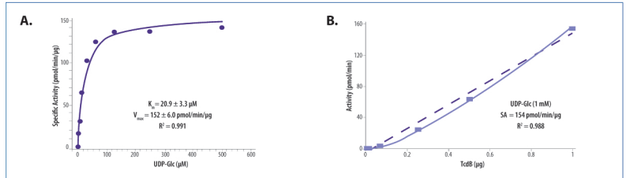



**FIGURE 2 TcdB assayed using CD39L3.** Each reaction was coupled to 0.1 µg of CD39L3. All reactions were carried out for 20 minutes at 37 °C. **(A)** Specific activity (SA) versus UDP-Glc. **(B)** Activity versus enzyme dose in the presence of 1 mM UDP-Glc.

### FIGURE 3

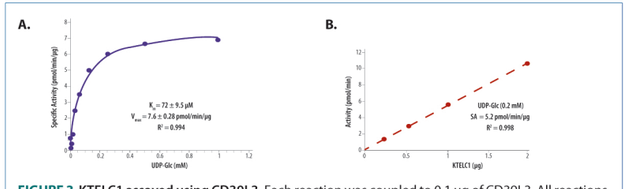



**FIGURE 3 KTELC1 assayed using CD39L3.** Each reaction was coupled to 0.1 µg of CD39L3. All reactions were carried out for 2 hours at 37 °C. **(A)** Specific activity (SA) versus UDP-Glc. **(B)** Activity versus enzyme dose in the presence of 0.2 mM UDP-Glc.

### FIGURE 4

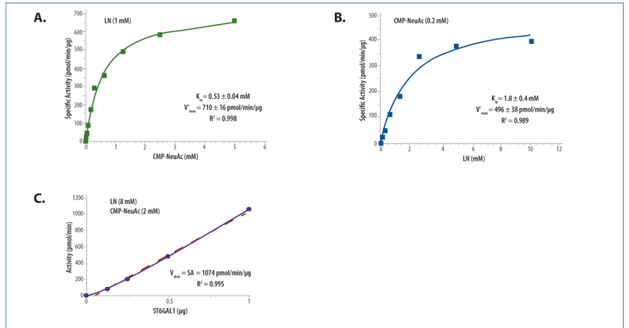



**FIGURE 4 ST6GAL1 assayed using CD73.** Each reaction was coupled to 0.1 µg of CD73. **(A)** Specific activity (SA) versus donor substrate CMP-NeuAc in the presence of 1 mM acceptor LN. **(B)** Specific activity versus acceptor substrate LN in the presence of 0.2 mM CMP-NeuAc. **(C)** Activity versus enzyme dose in the presence of 2 mM CMP-NeuAc and 8 mM LN.

**TABLE 1. Glycosyltransferases Assayed Using the Phosphatase-Coupled Method.**

| Glycosyltransferase[a] | Donor | Acceptor | Coupling Enzyme[a] | Specific Activity[b] (pmol/min/µg) | Buffer [c] & Temperature |
|---|---|---|---|---|---|
| TcdB | 1 mM UDP-Glc | $H_2O$ | CD39L3 | 154 | SB + 150 mM $K_2SO_4$, 23 °C |
| KTELC1 | 0.2 mM UDP-Glc | $H_2O$ | CD39L3 | 5.2 | 50 mM HEPES, 10 mM $MnCl_2$, 5 mM $CaCl_2$, pH 7.5, 37 °C |
| B4GALT1 | 1 mM UDP-Gal | 20 mM GlcNAc | CD39L3 | 1033 | SB, 23 °C |
| GALNT-1 | 1 mM UDP-GalNAc | 0.4 mg/mL MUC-1 | CD39L3 | 1967 | 100 mM Tris, 5 mM $MnCl_2$, 5 mM $CaCl_2$, pH 8.0, 23 °C |
| FUT-1 | 40 µM GDP-fucose | 15 mM Lactose | CD39L3 | 280 | 25 mM Tris, 10 mM $MnCl_2$, 5 mM $CaCl_2$, pH 7.5, 20% DMSO, 37 °C |
| FUT-3 | 40 µM GDP-fucose | 0.4 mM LN | CD39L3 | 73.5 | SB, 37 °C |
| FUT-5 | 40 µM GDP-fucose | 0.4 mM LN | CD39L3 | 98.8 | SB, 37 °C |
| FUT-7 | 80 µM GDP-fucose | 10 mg/mL Fetuin | CD39L3 | 314 | SB, 37 °C |
| FUT-8 | 64 µM GDP-fucose | $H_2O$ | CD39L3 | 10.6 | 0.1 M MES, pH 7.0, 23 °C |
| B3GNT6 | 200 µM UDP-GlcNAc | 0.6 mM pNP-α-GalNAc | CD39L3 | 75.0 ± 3.5 | SB + 20% DMSO, 37 °C |
| MGAT5 | 90 µM UDP-GlcNAc | 1 mM Biantennary N-linked core pentasaccharide | CD39L3 | 84.2 ± 5.6 | Buffer 1: 25 mM MES, pH 6.0, 37 °C. Buffer 2: 100 mM Tris, 5 mM $CaCl_2$, pH 7.5, 23 °C |
| ST6GalNAc2 | 500 µM CMP-Sialic acid | 20 mg/mL Asialofetuin | CD73 | 773 | SB, 37 °C |
| ST6GAL1 | 2 mM CMP-NeuAc | 8 mM LN | CD73 | 1074 | SB, 23 °C |

[a] All enzymes were expressed by R&D Systems.
[b] B3GNT6 and MGAT5 showed a synergistic enzymatic dose curve and their activities were determined based on triplicate single points. The specific activities for all of the other enzymes were determined based on the linear portions of corresponding enzyme dose curves with linear correlation coefficients > 0.98.
[c] SB, standard buffer of 25 mM Tris, 150 mM NaCl, 5 mM $MgCl_2$ and 5 mM $MnCl_2$ at pH 7.5. A decoupled assay was performed due to the buffer incompatibility of MGAT5 and CD39L3. The glycosyltransferase reaction was carried out in buffer 1. The phosphatase reaction was carried out in buffer 2.

## CONCLUSION

1. This method is universal to all glycosyltransferases that generate leaving groups with a removable phosphate.
2. The assay is quantitative and allows accurate kinetic analysis.
3. It is a convenient and cost effective assay because no separation or radioisotope is required.
4. The assay can be performed in a 96-well plate and provides a platform for high-throughput drug screening.