UDP-Glo™ detection protocol

River Stone has developed two protocols for determination of the UDP-glycosyl transferase activity

For initial screening we recommend using a plate reader assay such as the Promega "UDP-Glo™ Glycosyl-transferase Assay". This requires a plate reader for the measurement of luminescence.

The UDP-GloTM detection protocol is a faster protocol but it can lead to false positive.

Confirmation of initial hits, proper quantification of activity and structure elucidation will then need HPLC or LC-MS analysis.

Detailed protocols are provided with the kit, and online and phone support is available.

Both protocols are available on our website

www.gly-kit.com

GLY-KIT

Improving Small Molecules

UDP-Glo™ Glycosyltransferase detection assay protocol (Promega)

Step 1

After the UGT activity assay has been completed, equilibrate the assay/UDP standard plates to room temperature. During equilibration prepare the UDP-Glo detection reagents as described below.

Step -2

Prepare the UDP detection reagent

Calculate the required volumes of each reagent needed for your experiment and increase of decrease the volumes appropriately. Prepare 100mL, which will be sufficient for 440 wells (25µL /well)

Nucleotide Detection Reagent preparation

1. Equilibrate the Nucleotide Detection Buffer and ATP Detection Substrate to room temperature before use.
2. Transfer the entire volumes of Nucleotide Detection Buffer into the amber bottle containing ATP Detection Substrate to reconstitute the lyophilized luciferase enzyme/substrate mixture. This forms the Nucleotide Detection Reagent.
3. Mix to homogeneity by gently vortexing, swirling or by inverting the contents. The ATP Detection Substrate should go easily into solution in less than 1 minute.
4. Use Nucleotide Detection Reagent immediately or dispense into aliquots at less than 1 minute.

UDP Detection Reagent Preparation

The following instructions will prepare 1-30 ml of UDP Detection Reagent

1. Equilibrate an aliquot of Nucleotide Detection Reagent at room temperature.
2. Prepare 300µL of UDP-Glo™ working solution by adding 4µL of UDP-Glo™ Enzyme to 296µL Enzyme dilution buffer. Mix well.
3. Prepare UDP Detection Reagent by adding 10µL of UDO-Glo™ working solution to each 1ml of Nucleotide Detection Reagent immediately before use. Prepare 11 ml in total.
4. Mix content to homogeneity by gently pipetting or vortexing.

Notes

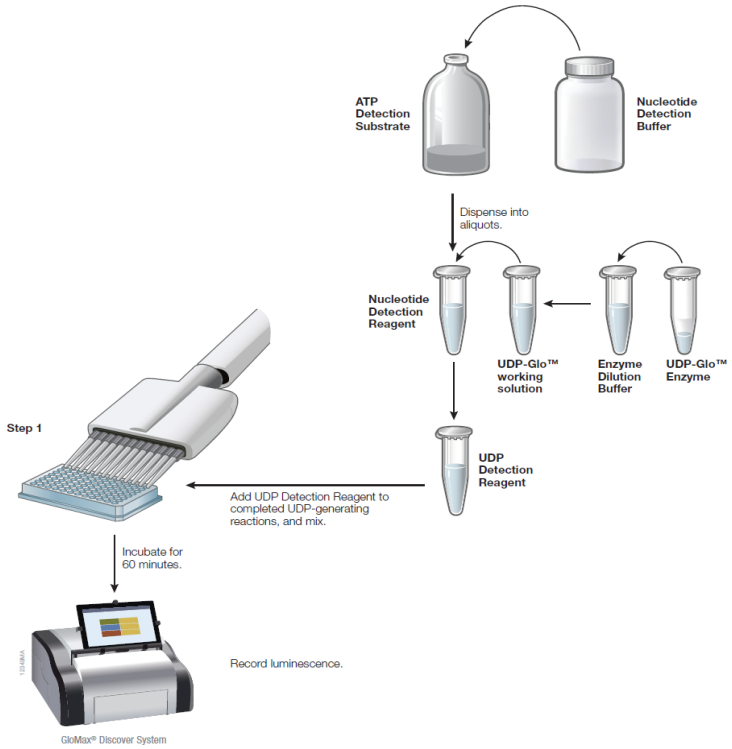
1. Make only enough UDP Detection Reagent required for the experiment. Return the remaining UDP-Glo™ Enzyme to less than -65°C.
2. To prepare more than 30ml of UDP Detection Reagent, increase the volume of UDP-Glo™ working solution by adjusting volumes of both the UDP-Glo™ Enzyme and Enzyme Dilution Buffer to accommodate the volume of UDP Detection Reagent needed for your experiment.
3. Because there is sufficient UDP-Glo™ Enzyme and Enzyme Dilution Buffer for the numbers of reactions listed for the assay size, discard any unused UDO-Glo™ working solution.

Step 3

Add 25µL UDP Detection Reagent to each well of the room temperature-equilibrated assay plates (including the plate containing the standard curve/additional controls).

Mix assay plate with a plate shaker for 30sec (use the GloMax machine for this), incubate for 1h at room temperature.

Measure the luminescence with the GloMax plate reader (Integration time: 0.25sec/well).



Contact Us

River Stone Biotech ApS
Fruebjergvej 3
2100 Copenhagen
Denmark

Contact:
contact@rstbio.com

Call
EU: +353 (0)87 184 1966
USA: +1 (703) 999-3537

What is Gly-Kit

The Gly-Kit platform is a library of 380 diverse "Family 1" UDP-glucose-dependent glycosyltransferase enzymes (UGTs) plus associated screening, analytical and lab scale production protocols ("Family 1" denotes glycosyltransferases that will glycosylate small molecules).

All the enzymes in Gly-Kit are found in plants (which have diverse UGTs to work with the diverse range of small molecules that occur in plants or their environment). The kit contains enzymes from all known Family 1 UGT sub-families and sub-sub-families and from a huge set of evolutionarily diverse plants.

The majority of the enzymes will be able to add glucose to small molecule substrates with relevant functional groups. Some enzymes will work with other sugars (such as xylose, rhamnose, galactose or glucuronic acid). We can advise you on the best path for specific sugars.

We realize that this may be your first step in determining if Gly-Kit will be able to help you in your current project. We are happy to assist you in determining if Gly-Kit is the right fit.

To discuss your order, or for more help, just get in touch. We would like to make sure Gly-Kit is a proper fit for your current goals. Once we connect and assure Gly-kit is the right fit, we will send you pricing options. Email us directly at sales@rstbio.com

To call us directly EU: +353 (0)87 184 1966 USA: +1 (703) 999-3537