HPLC 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-Glycosyl-transferase HPLC detection protocol

20 µL total volume of reactions
100 mM Tris-HCl buffer (pH 7.4)
1.25 mM UDP-glucose (UDPG) (a less pure quality than the one needed for the UDP-Glo™ assay may be used)
0.5 mM TCP or aglucone substrate of interest
Incubate overnight at 30 °C. Carry out the reactions in 96-well microtiter plates

Reaction setup for one assay reaction

Purified UGT enzyme[a]	5	µl
25 mM TCP or substrate of interest	0.4	µl
1 M Tris-HCl pH 7.4	2	µl
Milli-Q H2O	11.9	µl
FastAP phosphatase (1U/µL)[b]	0.2	µl
50 mM UDPG	0.5	µl
Total Volume	20	µl

[a]: Purified UGT enzyme is supplied in 10 mM Tris-HCl, 250 mM Imidazol, pH 7 and 50 % (v/v) Glycerol.
[b]: FastAP phosphatase (1U/µL) is from ThermoFischer Scientific (catalog no. EF0651)

DAY 1

Set up the assay reactions following the scheme above. Seal the 96-well microtiter plate with an adhesive seal and incubate overnight at 30 ˚C.

DAY 2

Terminate assay reactions with 3 volumes (60 µL) of ice-cold 100 % methanol and mix well by pipetting. Transfer the assay reactions to a spin filter plate (MSHVN45, Millipore) mounted on a collection plate (96-well plate). Ensure that the spin plate sits well or secure it with a little piece of tape. Centrifugate the plate at 3000 rpm for 3 min. Seal the sample collection plate and proceed with HPLC analysis.

Note

Depending on the substrate of interest that needs to be tested, it might be necessary to optimize the amounts of UGT enzyme, substrate concentration and reaction time in order to achieve optimal performance. For any question please contact us.

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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