UDP-Glo™ 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 assay for plate reader detection (Promega)

This protocol is based on the "UDP-Glo Glycosyltransferase Assay" protocol from Promega.
Please refer to: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/udp-glo-glycosyltransferase-assay-protocol.pdf

Prepare and assemble all UGT reactions on ice!

A.

Prepare a "4xReaction buffer": 200mM Tris-HCl, pH 7.4, 32mM MgCl2. Prepare 50mL in a falcon tube. Unused buffer can be stored at 4°C.

4xReaction buffer

Tris-HCl, pH 7.4 (1M Stock)	10mL	
$MgCl_2$ (2M Stock)	0.8mL	
MilliQ H_2O		add to 50mL

B.

Prepare 8mL of "Substrate mix" (this is enough for 4x 96-well assay plates, i.e. one screening round with the complete library

Substrate mix

4xReactionbuffer	1000µL	
100mM UDP-Glucose* (Ultrapure from Promega, V7091)	10µL	
1mM 2,4,5-Trichlorophenol (TCP)** (or substrate of interest)	500µL	
MilliQ H_2O		6490µL

Keep on ice until ready to use.

*Ultrapure UDP-Glucose should be used. Other sources of UDP-sugar may contain free UDP that could result in high background.
**TCP can be used as a positive control or as a substrate to test the performance of the library. UGTs active against TCP are located on GLY plate 1 & 2.

C.

Prepare 200µL of a 25µM UDP solution using the 10mM UDP standard (Promega):

25µM UDP solution

10mM UDP standard (Promega)	0.5µL
4XReaction buffer	20µL
MilliQ H_2O:	179.5µL

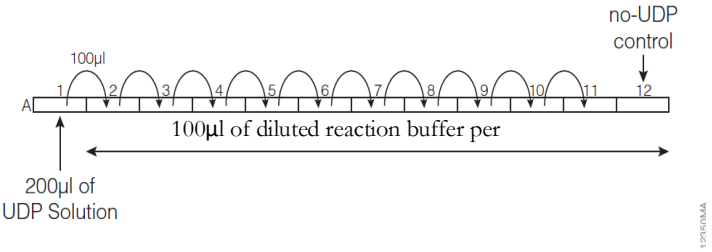
Add all of the **25µM UDP solution** to well A1 of a preparative 96-well plate.

D.

Dilute the 4xReaction buffer 10 times by adding 1800µL MilliQ H2O to 200µL of 4xReaction buffer. Dispense hereafter 100µL of the diluted Reaction buffer to wells A2 through A12 of the preparative 96-well plate.

E.

Perform a serial twofold dilution, as shown below, by transferring 100µL from well A1 to A2, pipetting to mix. Transfer 100µL from well A2 to A3, pipetting to mix. Repeat for wells A4 through A11. Discard the extra 100µL from well A11. Do not add UDP to the no-UDP control reaction in well A12.



5. Dilution scheme for creating a UDP standard curve.

F.

To generate a "UDP standard curve": Transfer 25µL of each UDP standard from the preparative 96-well plate to the first row (well A1 to A12) on a white 96-well plate for plate reader measurements. This plate will be called a "standard plate".

Standard plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD	STD	STD	STD	STD	STD	STD	STD	STD	STD	STD	STD
B												
C												
D												
E												
F												
G												
H												

G.

In 4 separate white 96-well plates dispense 20µL of **substrate mix** to all wells:

Assay plates 1-4:

	1	2	3	4	5	6	7	8	9	10	11	12
A	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
B	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
C	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
D	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
E	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
F	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
G	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL
H	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL	20µL

H.

Add 5µL UGT enzyme from the RST_GLY_Library (4 plates in total) to assay plates 1-4, gently pipetting to mix. Seal the plates (also the plate containing the standard curve) with an adhesive seal and incubate at 30 °C for 2-24h.

Reaction assay conditions are:

Assay volume = 25 µL
20mM Tris-HCl, pH 7.4 (final conc.)
3.2mM $MgCl_2$ (final conc.)
0.1mM UDP-Glucose (final conc.)
0.05mM Substrate (final conc.)
5 µL UGT enzyme

Plate layout of UGT reactions:

Assay plate 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pGLY1	pGLY2	pGLY3	pGLY4	pGLY5	pGLY6	pGLY7	pGLY8	pGLY9	pGLY10	pGLY11	pGLY12
B	pGLY13	pGLY14	pGLY15	pGLY16	pGLY17	pGLY18	pGLY19	pGLY20	pGLY21	pGLY22	pGLY23	pGLY24
C	pGLY25	pGLY26	pGLY27	pGLY28	pGLY29	pGLY30	pGLY31	pGLY32	pGLY33	pGLY34	pGLY35	pGLY36
D	pGLY37	pGLY38	pGLY39	pGLY40	pGLY41	pGLY42	pGLY43	pGLY44	pGLY45	pGLY46	pGLY47	No Enzyme
E	pGLY48	pGLY49	pGLY50	pGLY51	pGLY52	pGLY53	pGLY54	pGLY55	pGLY56	pGLY57	pGLY58	pGLY59
F	pGLY60	pGLY61	pGLY62	pGLY63	pGLY64	pGLY65	pGLY66	pGLY67	pGLY68	pGLY69	pGLY70	pGLY71
G	pGLY72	pGLY73	pGLY74	pGLY75	pGLY76	pGLY77	pGLY78	pGLY79	pGLY80	pGLY81	pGLY82	pGLY83
H	pGLY84	pGLY85	pGLY86	pGLY87	pGLY88	pGLY89	pGLY90	pGLY91	pGLY92	pGLY93	pGLY94	pGLY95

Assay plate 2:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pGLY96	pGLY97	pGLY98	pGLY99	pGLY100	pGLY101	pGLY102	pGLY103	pGLY104	pGLY105	pGLY106	pGLY107
B	pGLY108	pGLY109	pGLY110	pGLY111	pGLY112	pGLY113	pGLY114	pGLY115	pGLY116	pGLY117	pGLY118	pGLY119
C	pGLY120	pGLY121	pGLY122	pGLY123	pGLY124	pGLY125	pGLY126	pGLY127	pGLY128	pGLY129	pGLY130	pGLY131
D	pGLY132	pGLY133	pGLY134	pGLY135	pGLY136	pGLY137	pGLY138	pGLY139	pGLY140	pGLY141	pGLY142	No Enzyme
E	pGLY143	pGLY144	pGLY145	pGLY146	pGLY147	pGLY148	pGLY149	pGLY150	pGLY151	pGLY152	pGLY153	pGLY154
F	pGLY155	pGLY156	pGLY157	pGLY158	pGLY159	pGLY160	pGLY161	pGLY162	pGLY163	pGLY164	pGLY165	pGLY166
G	pGLY167	pGLY168	pGLY169	pGLY170	pGLY171	pGLY172	pGLY173	pGLY174	pGLY175	pGLY176	pGLY177	pGLY178
H	pGLY179	pGLY180	pGLY181	pGLY182	pGLY183	pGLY184	pGLY185	pGLY186	pGLY187	pGLY188	pGLY189	pGLY190

Assay plate 3:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pGLY191	pGLY192	pGLY193	pGLY194	pGLY195	pGLY196	pGLY197	pGLY198	pGLY199	pGLY200	pGLY201	pGLY202
B	pGLY203	pGLY204	pGLY205	pGLY206	pGLY207	pGLY208	pGLY209	pGLY210	pGLY211	pGLY212	pGLY213	pGLY214
C	pGLY215	pGLY216	pGLY217	pGLY218	pGLY219	pGLY220	pGLY221	pGLY222	pGLY223	pGLY224	pGLY225	pGLY226
D	pGLY227	pGLY228	pGLY229	pGLY230	pGLY231	pGLY232	pGLY233	pGLY234	pGLY235	pGLY236	pGLY237	No Enzyme
E	pGLY238	pGLY239	pGLY240	pGLY241	pGLY242	pGLY243	pGLY244	pGLY245	pGLY246	pGLY247	pGLY248	pGLY249
F	pGLY250	pGLY251	pGLY252	pGLY253	pGLY254	pGLY255	pGLY256	pGLY257	pGLY258	pGLY259	pGLY260	pGLY261
G	pGLY262	pGLY263	pGLY264	pGLY265	pGLY266	pGLY267	pGLY268	pGLY269	pGLY270	pGLY271	pGLY272	pGLY273
H	pGLY274	pGLY275	pGLY276	pGLY277	pGLY278	pGLY279	pGLY280	pGLY281	pGLY282	pGLY283	pGLY284	pGLY285

Assay plate 4:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pGLY286	pGLY287	pGLY288	pGLY289	pGLY290	pGLY291	pGLY292	pGLY293	pGLY294	pGLY295	pGLY296	pGLY297
B	pGLY298	pGLY299	pGLY300	pGLY301	pGLY302	pGLY303	pGLY304	pGLY305	pGLY306	pGLY307	pGLY308	pGLY309
C	pGLY310	pGLY311	pGLY312	pGLY313	pGLY314	pGLY315	pGLY316	pGLY317	pGLY318	pGLY319	pGLY320	pGLY321
D	pGLY322	pGLY323	pGLY324	pGLY325	pGLY326	pGLY327	pGLY328	pGLY329	pGLY330	pGLY331	pGLY332	No Enzyme
E	pGLY333	pGLY334	pGLY335	pGLY336	pGLY337	pGLY338	pGLY339	pGLY340	pGLY341	pGLY342	pGLY343	pGLY344
F	pGLY345	pGLY346	pGLY347	pGLY348	pGLY349	pGLY350	pGLY351	pGLY352	pGLY353	pGLY354	pGLY355	pGLY356
G	pGLY357	pGLY358	pGLY359	pGLY360	pGLY361	pGLY362	pGLY363	pGLY364	pGLY365	pGLY366	pGLY367	pGLY368
H	pGLY369	pGLY370	pGLY371	pGLY372	pGLY373	pGLY374	pGLY375	pGLY376	pGLY377	pGLY378	pGLY379	pGLY380

Note: “No Enzyme control” wells will be used as background reference. It is recommended to also include a “No substrate control” for each UGT enzyme: Though only rarely an issue glycerol present as cryoprotectant in the enzyme solution can potentially be glucosylated, and this may lead to background luminescence in the assay. One may circumvent this by applying less UGT enzyme in the assay, extending the reaction incubation time.

I.

After UGT assay completion proceed with the UDP-GLO Glycosyltransferase detection assay protocol (Promega). It is recommended to perform the detection assay immediately after UGT assay termination to avoid any reduction of UDP-GLO Glycosyltransferase detection signal.

End of protocol.

Contact Us

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What is Gly-Kit

The Gly-Kit platform is a library of 380 diverse “Family 1” UDP-glucose-
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.
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