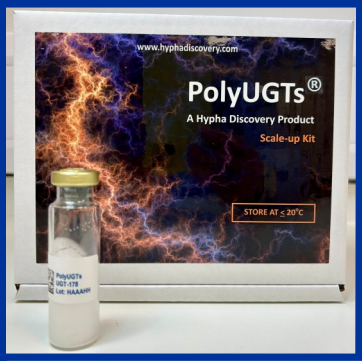


PolyUGTs scale-ups - tips and tricks

Glucuronides formed in PolyUGT screening reactions can be scaled up using PolyUGT scale-up kits, containing both enzyme and UDPGA.

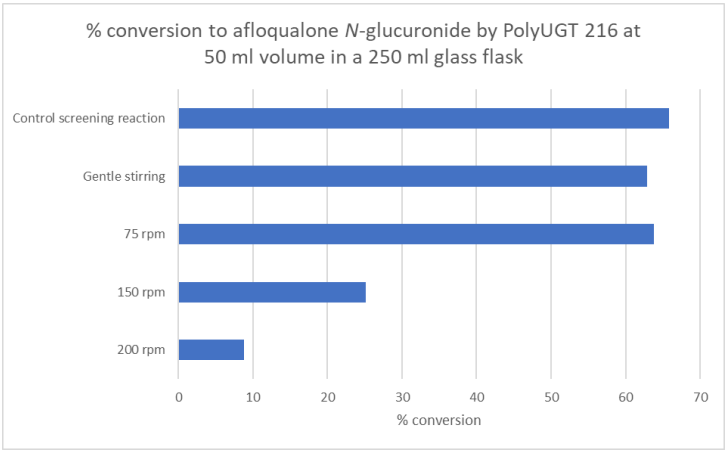
Each scale-up vial provides x20 enzyme than that provided in the screening kit. Sufficient UDPGA is provided for a 5mM incubation.

There is the option to optimise the reaction before scale-up.

Shaking speed

The recommended scale-up format is simply to incubate the reaction in a gently swirling glass flask, either on an orbital shaker set at a low speed, or with a slowly rotating stirrer bar. Static incubation is also possible, with intermittent swirling. Refer to the instructions for more details. High shaking speeds will likely have a detrimental effect.

SLOW MIXING = BETTER RESULTS

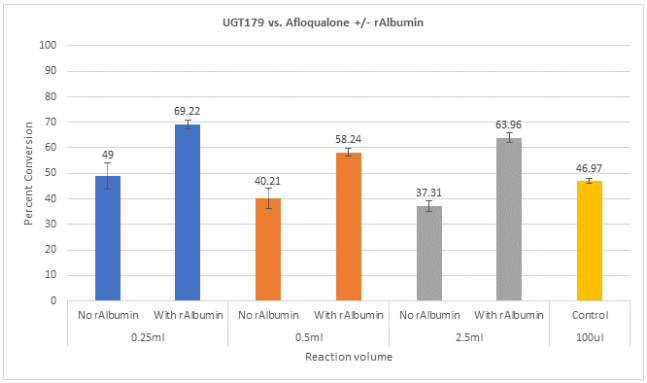


Use of 24 well blocks

Increasing reaction volume in the 24 well plastic blocks from 0.25 ml to 2.5 ml can result in a decrease in activity of up to 20%, depending on the substrate and isoform.

Addition of albumin

Addition of rAlbumin (recombinant bovine serum albumin from NEB, meat-free), standard BSA or HSA to give 0.1 mg/ml in the reaction can help to prevent any non-specific binding of the UGT protein to reaction vessels, especially where plastic is being used. The effect is isoform and substrate dependent.

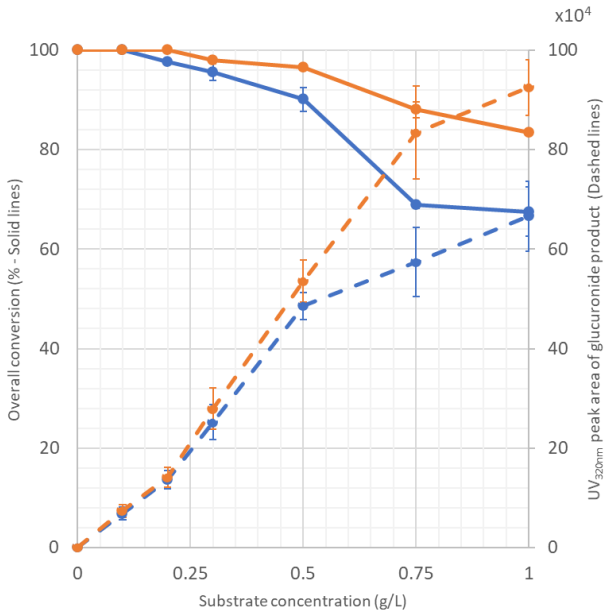


Increasing substrate concentration

Increasing the substrate concentration from the 100 mg/L used in the screening reaction to generate more product is possible, but is isoform and substrate dependent.

Increasing UDPGA concentration

Increasing the concentration of UDPGA in the reaction from the 5 mM concentration used in the screening reaction can improve conversion, especially when paired with an increase in substrate concentration. Note that in some reactions addition of more UDPGA can lead to over-metabolism to e.g. diglucuronides. UDPGA is expensive so this should be used judiciously.



Dose escalation up to 1 g/L 4-methylumbelliferone with either 5 mM (**blue**) or 10 mM (**orange**) UDPGA and 1 µM PolyUGT