

UGT Reaction Mix Solution A (UDPGA Cofactor)

Catalog Number.....451300 Storage Conditions.....STORE AT -20°C

Lot Number.....4323001 Date Released.....2014 December

Package Size.....2 mL

Solution A Components...25 mM Uridine 5'-Diphospho-Glucuronic Acid (UDPGA) in water.

Stability:

This product showed no loss in performance after 10 freeze/thaw cycles. Long term storage stability is under investigation.

Description and Suggested Use:

The measurement of glucuronidation activity catalyzed by microsomes, S9 and recombinant UGT enzymes requires an appropriate incubation buffer system containing UDPGA, an essential enzyme cofactor. The Corning® GENTEST "UGT Reaction Mix" consists of two reagents, Solution A (25 mM UDPGA) and Solution B (5X-UGT Assay Buffer with Alamethicin). Each reagent is sold separately. Combined they support glucuronidation by microsomes, S9 and recombinant UGT enzymes.

For a suggested UGT-glucuronidation activity assay, the final concentration of components are listed in the table below. Under these conditions, Solution A is a 12.5X concentrate for a 2 mM final UDPGA concentration and Solution B is a 5X concentrate.

Reagent	Final Concentration
Tris-HCl (pH 7.5)	50 mM
MgCl ₂	8 mM
Alamethicin	25 µg/mL
UDPGA	2 mM
HLM or recombinant UGT	Determined by end user, typically 0.1 to 1.0 mg/mL
Substrate and concentration	Determined by end user
Purified water	To volume
Methanol	0.25% (used to solubilize the alamethicin in preparing this product)

Solution B contains alamethicin which reduces latency in UGT activity (Fisher et.al., DMD, 28, 560-566, 2000). Alamethicin is a fungal derived peptide that can form pores in membranes (Little et.al., DMD, 25, 5-11, 1997). Alamethicin, unlike detergents which have also been used to reduce UGT latency, is not known to inhibit or denature other enzymes such as P450s. The addition of alamethicin stimulates UGT activity. The degree of stimulation depends on substrate, enzyme and source.]

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