

Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798
info@dls.com
<https://www.dls.com>

Gentest™ UGT Reaction Mix

Solution B (5X-UGT Buffer Mix with Alamethicin)

Catalog Number.....451320

Storage Conditions.....STORE AT -20°C

Lot Number.....2404031

Date Released.....2024 May

Package Size5 mL

Solution B Components.....250 mM Tris-HCl, 40 mM MgCl₂, 0.125 mg/mL Alamethicin in water.

Stability:

This product showed no loss in performance after 10 freeze/thaw cycles.

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 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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Safety Recommendations:

Safety assessment indicates this product is non-hazardous; therefore no SDS [Safety Data Sheet] is provided. Handle in accordance with good industrial hygiene and laboratory safety practices.



18 June 2024

Quality Assurance

Date