Discovery Labware, Inc.
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CORNING

0.5M Potassium Phosphate Buffer, pH 7.4

P450/Phase II Drug Metabolism Assay Buffer

Catalog Number: 451201
Quantity: 500mL
Lot Number: 8352001

Storage Conditions: Room Temperature
Date Released: 2018 December

Molarity: 0.5M
pH: 7.4

Product has been filter sterilized (0.2 µ filtration).
Product has been tested and found to be free of bacteria and fungi.

Certified for use in P450 enzyme assays and Phase II drug metabolism enzyme assays.
Qualification Assay: Testosterone 6β-Hydroxylase with CYP3A4 Corning® Supersomes™ Enzyme (456202).

METHOD: A 0.50 ml reaction mixture containing 10 pmole P450, 1.3mM NADP+, 3.3mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3mM magnesium chloride and 0.2mM testosterone in 100mM potassium phosphate (pH 7.4) was incubated at 37°C for 10 min. After incubation, the reaction was stopped by the addition of 250 µl acetonitrile and centrifuged (10,000 x g) for 3 minutes. 100 µl of the supernatant was injected into a 4.6 x 250 mm 5µ C18 HPLC column and separated at 45°C with a mobile phase initially of 58% methanol increasing to 62% methanol over 8 min. and at a flow rate of 1.0 ml per min. The product was detected by its absorbance at 254 nm (242 nm is optimal) and quantitated by comparing the absorbance to a standard curve of 6β-hydroxytestosterone.

Recommended Use:

- Use with animal and human tissue fractions (e.g. cytosol, S9 and microsomes) or recombinant enzymes (e.g. Corning Gentest™ P450, UGT and MAO-A/B). See complete list of Corning Gentest products that can be assayed with this buffer.
- KPO4 buffer is highly recommended for most P450 assays (microsomal or recombinant enzymes) with the exception of CYP 2C9 and 2A6 where a Tris buffer system is more appropriate.
- KPO4 buffer is recommended for UGT reactions in microsomal systems.
- For most assays involving Phase I and Phase II drug metabolism enzymes (e.g. P450, UGT and MAO) a final KPO4 buffer concentration of 0.1 M is recommended. The optimum buffer concentration may vary depending on the specific enzyme being tested.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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- The 0.5 M KPO4 buffer can be combined with Corning® Gentest™ NADPH re-generating system (Solution A, Cat# 451220 and Solution B, Cat# 451200) to make a convenient assay mix for measuring enzymes requiring NADPH co-factor. Solution A is a 20X concentrate of NADP+, Glucose 6-phosphate (G-6P) and $MgCl_2$. Solution B is a 100X concentrate of Glucose 6-phosphate dehydrogenase (G-6PDH). The chart below is an example showing how the 3 products can be combined to make a standard P450 assay mix.

Assay Reagent	Volumes (µl) for 400 µl incubation volume	Final Concentrations	Volumes for 10x 400 µl reactions
0.5 M KPO4 (451201)	80	100 mM	800
Solution A (451220) (20x)	20	1.3 mM NADP, 3.3. mM G-6P, 3.3 MgCl	200
Solution B (451200) (100x)	4	0.4 Units/ml G-6PDH	40
H2O	278	------	2780
[1] 10 mM Substrate (dissolved in Acetonitrile)	8	0.2 mM	80
[2]Liver microsomes (20 mg/ml)	10 (added to 390 µl of assay mix)	0.1 mg/ml	10 (added to 390 µl of assay mix)

[1] The final acetonitrile concentration contributed by the substrate is 2%. Acetonitrile can inhibit P450 reactions at concentrations greater than 2% (Chauret et al. (1998) Drug Metab Dispos. **26**: 1-4and Busby et al., (1999) *Drug Metab. Dispos.* **27**, 246-249). See our Web-site (http://www.corning.com/lifesciences).

[2]We recommend mixing all components and adding enzyme last to initiate the reaction.

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.

Quality Assurance

21 Dec, 2018
Date