Discovery Labware, Inc.
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Tel: 1.978.442.2200 (U.S.)
CLSTechServ@Corning.com

www.corning.com/lifesciences

CORNING

0.5M Potassium Phosphate Buffer, pH 7.4

P450/Phase II Drug Metabolism Assay Buffer

Catalog Number: 451201
Quantity: 500mL
Lot Number: 3352912

Storage Conditions: Room Temperature
Date Released: 2014 February

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 BD 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.

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SAFETY RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

Do not eat, drink or smoke.

Avoid contact with skin or eyes.

Do not inhale aerosols.

Do not pipette by mouth.

Wear suitable protective clothing, gloves and eye protection.

Steam sterilize product or treat product with a 1% solution of sodium hypochlorite prior to disposal.