Recombinant Cytochrome P450s Canine Bactosomes® Enzymes

Product No.	Description
CYP300	Canine CYP1A1LR BACTOSOMES Enzymes
CYP301	Canine CYP2B11LR BACTOSOMES Enzymes
CYP302	Canine CYP2C21LR BACTOSOMES Enzymes
CYP303	Canine CYP2C41LR BACTOSOMES Enzymes
CYP304	Canine CYP2D15LR BACTOSOMES Enzymes
CYP305	Canine CYP3A12LR BACTOSOMES Enzymes
CYP306	Canine CYP3A26LR BACTOSOMES Enzymes
CYP307	Canine CYP1A2LR BACTOSOMES Enzymes
CYP308	Canine CYP1B1LR BACTOSOMES Enzymes

Product Description:

BACTOSOMES enzymes are *E. coli* membrane preparations containing recombinant canine cytochrome P450 and co-expressed canine NADPH P450 reductase. The level of reductase relative to the CYP influences the activity and time for which the reaction is linear. The higher the relative level of reductase, the higher the activity however the time for which the reaction is linear reduces.

Storage: **≤ -80°C**

Materials

200 mM potassium phosphate pH 7.4
100 mM $MgCl_2$
Deionized water
Substrate solution
NADP+
Glucose-6-phosphate disodium salt
Glucose-6-phosphate dehydrogenase
1 M HCl, Methanol or acetonitrile as stop reagent

Equipment

Water bath set to 37°C
Suitable polypropylene vials
Centrifuge

Incubation Procedure:

BACTOSOMES enzymes require either NADPH or a NADPH regenerating system for activity. Incubations are usually conducted in 50 or100 mM potassium phosphate buffer, but other buffers

may be used. Some CYP isoforms (CYP4A11) require a specific buffer, check the data sheet accompanying the product for details.

DRUG METABOLISM

1) Thaw frozen BACTOSOMES enzymes on ice. Once thawed, keep the vial of BACTOSOMES enzymes on ice and use as soon as possible after thawing.
2) Prepare NADPH Regenerating System (NB; this is a 5x stock, calculate the volume required accordingly, eg. for a 1 ml reaction 200 µl would be added to 800 µl premix).
   a) Dissolve 4.2 mg $NADP^+$ and 7.1 mg glucose-6-phosphate disodium salt in 1 ml 50 mM potassium phosphate pH 7.4 (adjust the amounts according to the volume required).
   b) Add 5 U glucose-6-phosphate dehydrogenase from S. cerivisiae to the solution
3) Prepare the following premix on ice (sufficient for 25 x 0.2 ml reactions), 4 ml total volume. When calculating final concentrations for substrate and cytochrome P450 the premix will be diluted 1.25 times by the addition of NADPH generating system.

For reactions in 50 mM potassium phosphate:

200 mM potassium phosphate pH 7.4	1000 µl
100 mM $MgCl_2$	250 µl
Water	to 4000 µl
Substrate / test compound	to 1.25 X required final concentration
BACTOSOMES enzymes	to 1.25 X required final CYP concentration

For reactions in 100 mM potassium phosphate:

200 mM potassium phosphate pH 7.4	2250 µl
100 mM $MgCl_2$	250 µl
Water	to 4000 µl
Substrate / test compound	to 1.25 X required final concentration
BACTOSOMES enzymes	to 1.25 X required final CYP concentration

The volume of substrate will be determined by the required final concentration. Solvent concentration (e.g. methanol, DMSO) should be kept to a minimum with a maximum concentration in the assay of 1%. We try and keep it below 0.1%.

The concentration of cytochrome P450 will be dependent on the requirements of the assay and the activity of the enzyme with the substrate being used. Typical cytochrome P450 concentrations can be found on the data sheet accompanying the specific Bactosome product being used. It should be borne in mind, however, that these concentrations are specific to the substrate being used and are set to minimize substrate loss (less than 10% across the assay). If you are looking for substrate loss in the assay then the concentration of cytochrome P450 should be increased accordingly.

4) Warm the NADPH Regeneration system to 37°C. Add 160 µl premix to each assay tube (1.5 ml polypropylene microtubes work well) and incubate at 37°C for 5 min. The assay volume can be adjusted as required, we also use 1 ml final volume assays in which case 800 µl of premix would be used per tube in 15 ml polypropylene conical tubes.
5) Add 40 µl NADPH Regeneration system (200 µl for a 1 ml assay volume) and incubate at 37°C (usually 5 - 15 minutes for high reductase with a good substrate and 15 – 40 mins for low reductase).
6) Stop the reaction by the addition of one of;
   0.1 volumes 1 M HCl (50 mM potassium phosphate assay)
   0.125 volumes 1 M HCL (100 mM potassium phosphate assay)
   0.5 volumes acetonitrile
   1 volume methanol
7) Incubate the samples on ice for 10 minutes and then centrifuge; approx 14,000 rpm for 10 mins for microtubes or 4,000 rpm for 20 mins for 15 ml tubes)
8) Recover the supernatants for further analysis.

CAUTION:

This product is being sold for research and/or manufacturing purposes only. The biological samples supplied by BioIVT, or any material isolated from the samples, are for in-vitro research use only and are not to be used as a source of material for clinical therapies. Human material may be used in vivo in animals. The user assumes all responsibility for its usage and disposal, in accordance with all regulations.