Recombinant Cytochrome P450s Human EasyCYP™ BACTOSOMES® Enzymes

Product No.	Description
CYP-EZ001	CYP1A2R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ002	CYP3A4R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ003	Control BACTOSOMES Enzymes
CYP-EZ005	CYP3A4BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ006	CYP2C9R EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ007	CYP2D6R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ008	CYP2C19R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ009	CYP2E1R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ010	CYP3A4LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ011	CYP2A6R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ012	CYP1A2LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ013	CYP2D6LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ014	CYP1A1R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ015	CYP3A5LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ016	CYP2B6LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ017	CYP2C8R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ018	CYP1A1LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ019	CYP2C9HR EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ020	CYP2B6R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ021	CYP1B1*3LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ022	CYP2C18LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ023	CYP1B1LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ024	CYP1B1*4LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ025	CYP4A11LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ026	CYP4A11R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ027	CYP2D6*2R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ028	CYP2C19LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ029	CYP2D6*10R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ030	CYP2D6*39R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ031	CYP2C9*2R EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ032	CYP2A13LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ033	CYP2A13R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ034	CYP2J2LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ035	CYP3A4BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ036	CYP2E1BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ037	CYP2C9BHR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ038	CYP2C9BR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ039	CYP2C9*3R EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ040	CYP4F2BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ041	CYP2B6BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ042	CYP2C9*2BR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ043	CYP4A11BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5

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CYP-EZ044	CYP2C9*3BR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ045	CYP3A5BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ046	CYP3A5R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ047	CYP2C8LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ048	CYP3A5BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ049	CYP2C8BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ052	CYP2C8BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ055	CYP4F3BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ056	CYP4F3BR EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ057	CYP4F3BBLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ058	CYP4F3BBR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ059	CYP3A7R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ060	CYP3A7BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ061	CYP2B6BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ062	CYP2C19BLR EasyCYP BACTOSOMES Enzymes, Low-Reductase + b5
CYP-EZ063	CYP2C19BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ064	CYP2A6BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ065	CYP17A1LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ066	CYP17A1R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ067	CYP46A1LR EasyCYP BACTOSOMES Enzymes, Low-Reductase
CYP-EZ068	CYP46A1R EasyCYP BACTOSOMES Enzymes, High-Reductase
CYP-EZ069	CYP17A1BR EasyCYP BACTOSOMES Enzymes, High-Reductase + b5
CYP-EZ073	CYP2D6*17LR EasyCYP BACTOSOMES Enzymes, Low-Reductase

PRODUCT DESCRIPTION:

Human BACTOSOMES enzymes are available in EasyCYP format where both the CYP concentration and total protein concentration have been standardized at 1 nmol/ml and 10 mg/ml respectively for convenience. EasyCYP BACTOSOMES enzymes are *E. coli* membrane preparations containing recombinant human cytochrome P450 and co-expressed human NADPH P450 reductase. They are available with and without supplemented human cytochrome b_5. EasyCYP BACTOSOMES enzymes come with a choice of high (CYPxxxR (except CYP2C9)or CYPxxxHR (CYP2C9 only)) or low reductase (CYPxxxLR (except CYP2C9) or CYPxxxR (CYP2C9 only)). 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

EQUIPMENT

Water bath set to 37°C
Suitable polypropylene vials
Centrifuge

1 M HCl, Methanol or acetonitrile as stop reagent

INCUBATION PROCEDURE:

EasyCYP 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 EasyCYP BACTOSOMES enzymes on ice. Once thawed, keep the vial of EasyCYP 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 EasyCYP

BACTOSOMES enzyme product being used. Note, that these concentrations are specific to the substrate being used and are set to minimise 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.