

## RECOMBINANT CYTOCHROME P450s - HUMAN BACTOSOMES® ENZYMES

Product No.	Description
CYP001	CYP1A2R BACTOSOMES Enzymes, High-Reductase
CYP002	CYP3A4R BACTOSOMES Enzymes, High-Reductase
CYP003	Control BACTOSOMES Enzymes
CYP004	Reductase BACTOSOMES Enzymes, High-Reductase
CYP005	CYP3A4BR BACTOSOMES Enzymes, High-Reductase + b5
CYP006	CYP2C9R BACTOSOMES Enzymes, Low-Reductase
CYP007	CYP2D6R BACTOSOMES Enzymes, High-Reductase
CYP008	CYP2C19R BACTOSOMES Enzymes, High-Reductase
CYP009	CYP2E1R BACTOSOMES Enzymes, High-Reductase
CYP010	CYP3A4LR BACTOSOMES Enzymes, Low-Reductase
CYP011	CYP2A6R BACTOSOMES Enzymes, High-Reductase
CYP012	CYP1A2LR BACTOSOMES Enzymes, Low-Reductase
CYP013	CYP2D6LR BACTOSOMES Enzymes, Low-Reductase
CYP014	CYP1A1R BACTOSOMES Enzymes, High-Reductase
CYP015	CYP3A5LR BACTOSOMES Enzymes, Low-Reductase
CYP016	CYP2B6LR BACTOSOMES Enzymes, Low-Reductase
CYP017	CYP2C8R BACTOSOMES Enzymes, High-Reductase
CYP018	CYP1A1LR BACTOSOMES Enzymes, Low-Reductase
CYP019	CYP2C9HR BACTOSOMES Enzymes, High-Reductase
CYP020	CYP2B6R BACTOSOMES Enzymes, High-Reductase
CYP021	CYP1B1*3LR BACTOSOMES Enzymes, Low-Reductase
CYP022	CYP2C18LR BACTOSOMES Enzymes, Low-Reductase
CYP023	CYP1B1LR BACTOSOMES Enzymes, Low-Reductase
CYP024	CYP1B1*4LR BACTOSOMES Enzymes, Low-Reductase
CYP025	CYP4A11LR BACTOSOMES Enzymes, Low-Reductase
CYP026	CYP4A11R BACTOSOMES Enzymes, High-Reductase
CYP027	CYP2D6*2R BACTOSOMES Enzymes, High-Reductase
CYP028	CYP2C19LR BACTOSOMES Enzymes, Low-Reductase
CYP029	CYP2D6*10R BACTOSOMES Enzymes, High-Reductase
CYP030	CYP2D6*39R BACTOSOMES Enzymes, High-Reductase
CYP031	CYP2C9*2R BACTOSOMES Enzymes, Low-Reductase
CYP032	CYP2A13LR BACTOSOMES Enzymes, Low-Reductase
CYP033	CYP2A13R BACTOSOMES Enzymes, High-Reductase
CYP034	CYP2J2LR BACTOSOMES Enzymes, Low-Reductase
CYP035	CYP3A4BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP036	CYP2E1BR BACTOSOMES Enzymes, High-Reductase + b5
CYP037	CYP2C9BHR BACTOSOMES Enzymes, High-Reductase + b5
CYP038	CYP2C9BR BACTOSOMES Enzymes, Low-Reductase + 5
CYP039	CYP2C9*3R BACTOSOMES Enzymes, Low-Reductase
CYP040	CYP4F2BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP041	CYP2B6BR BACTOSOMES Enzymes, High-Reductase + b5
CYP042	CYP2C9*2BR BACTOSOMES Enzymes, Low-Reductase + b5
CYP043	CYP4A11BR BACTOSOMES Enzymes, High-Reductase + b5

CYP044	CYP2C9*3BR BACTOSOMES Enzymes, Low-Reductase + b5
CYP045	CYP3A5BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP046	CYP3A5R BACTOSOMES Enzymes, High-Reductase
CYP047	CYP2C8LR BACTOSOMES Enzymes, Low-Reductase
CYP048	CYP3A5BR BACTOSOMES Enzymes, High-Reductase + b5
CYP049	CYP2C8BR BACTOSOMES Enzymes, High-Reductase + b5
CYP052	CYP2C8BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP053	CYP3A7LR BACTOSOMES Enzymes, Low-Reductase
CYP054	CYP3A7BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP055	CYP4F3BLR BACTOSOMES Enzymes, Low-Reductase
CYP056	CYP4F3BR BACTOSOMES Enzymes, High-Reductase
CYP057	CYP4F3BBLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP058	CYP4F3BBR BACTOSOMES Enzymes, High-Reductase + b5
CYP059	CYP3A7R BACTOSOMES Enzymes, High-Reductase
CYP060	CYP3A7BR BACTOSOMES Enzymes, High-Reductase + b5
CYP061	CYP2B6BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP062	CYP2C19BLR BACTOSOMES Enzymes, Low-Reductase + b5
CYP063	CYP2C19BR BACTOSOMES Enzymes, High-Reductase + b5
CYP064	CYP2A6BR BACTOSOMES Enzymes, High-Reductase + b5
CYP065	CYP17A1LR BACTOSOMES Enzymes, Low-Reductase
CYP066	CYP17A1R BACTOSOMES Enzymes, High-Reductase
CYP067	CYP46A1LR BACTOSOMES Enzymes, Low-Reductase
CYP068	CYP46A1R BACTOSOMES Enzymes, High-Reductase
CYP069	CYP17A1BR BACTOSOMES Enzymes, High-Reductase + b5
CYP070	CYP26A1LR BACTOSOMES Enzymes, Low-Reductase
CYP071	CYP26B1LR BACTOSOMES Enzymes, Low-Reductase
CYP072	CYP51R BACTOSOMES Enzymes, High-Reductase
CYP073	CYP2D6*17LR BACTOSOMES Enzymes, Low-Reductase

**PRODUCT DESCRIPTION:**

BACTOSOMES enzymes are E. coli membrane vesicles containing a single recombinant human cytochrome P450 co-expressed with recombinant human NADPH P450 reductase. Each vial contains 1 nmol of CYP. They are available with and without supplemented human cytochrome b<sub>5</sub>. 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<sub>2</sub>
- Deionized water
- Substrate solution

**EQUIPMENT**

- Water bath set to 37°C
- Suitable polypropylene vials
- Centrifuge

NADP+  
Glucose-6-phosphate disodium salt  
Glucose-6-phosphate dehydrogenase  
1 M HCl, Methanol or acetonitrile as stop reagent

### INCUBATION PROCEDURE:

BACTOSOMES enzymes require either NADPH or a NADPH regenerating system for activity. Incubations are usually conducted in 50 or 100 mM potassium phosphate buffer, but other buffers may be used. *Note: 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. cerevisiae* 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 <sub>2</sub>	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 <sub>2</sub>	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.*