RECOMBINANT HUMAN CARBOXYLESTERASE (CES) BACTOSOMES® ENZYMES

Product No.	Description
CYP152	Carboxylesterase 1 (CES1) BACTOSOMES Enzymes
CYP153	Carboxylesterase 2 (CES2) BACTOSOMES Enzymes
CYP003	Control BACTOSOMES

PRODUCT DESCRIPTION:

Human Carboxylesterase (CES) BACTOSOMES enzymes are E-coli membrane preparations containing recombinant human Carboxylesterase and are supplied in 50 mM Tris-acetate, pH 7.6, containing 250 mM sucrose and 0.25 mM EDTA. Each vial of human CES BACTOSOMES enzymes contains 1 mg bacterial membrane protein.

STORAGE: **≤ -80°C**

MATERIALS

50 mM potassium phosphate, pH 7.4 (assay buffer; also used for enzyme dilution)
Deionized water
Substrate solution
1 M HCl, methanol or acetonitrile as stop reagent

EQUIPMENT

Water bath set to 37°C
Suitable polypropylene tubes
Centrifuge

INCUBATION PROCEDURE:

Incubations are usually conducted in 50 mM potassium phosphate buffer, pH 7.4, although other buffers may be used.

DRUG METABOLISM

1) Thaw the CES and keep on ice once thawed. Mix gently just before use.
2) Prepare incubations on ice, using the guide below. Reactions can be initiated by the addition of either enzyme or substrate – whichever is being used to initiate the reaction should be omitted at the preparation stage. Multiple incubations should be prepared from a pre-mix (see below).

For a single 0.2 ml incubation:

200 mM potassium phosphate pH 7.4	50 µl
Water	(150 – x – y) µl
Substrate / test compound	x µl
CES	y µl

Pre-mix for 20 x 0.2 ml incubations:

200 mM potassium phosphate pH 7.4	1000 µl
Water	(3000 – xx – yy) µl
Substrate / test compound	xx µl
CES	yy µl

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% (v/v), if possible.

The concentration of CES will be dependent on the requirements of the assay and the activity of the enzyme with the substrate being used. Typical protein concentrations can be found on the data sheet accompanying the specific CES product being used. It should be borne in mind, however, 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 CES should be increased accordingly.

3) Add the appropriate volume of pre-mix (with either the substrate or the CES omitted) to each assay tube (1.5 ml polypropylene microtubes work well) and pre-incubate at 37°C for 5 min.
4) Initiate the reaction by adding either substrate or CES to each tube, and incubate at 37°C (typically 5 – 30 minutes, but this depends on the CES and substrate being used).
5) Stop the reaction(s) by the addition of one of the following:
   0.1 volumes 1 M HCl (20 µl for a 200 µl incubation)
   0.5 volumes acetonitrile (100 µl for a 200 µl incubation)
   1 volume methanol (200 µl for a 200 µl incubation)
6) Place the samples on ice for at least 10 minutes and then centrifuge: approximately 13,000 rpm for 10 mins for microtubes or 4,000 rpm for 20 mins for 15 ml tubes)
7) 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.