RECOMBINANT HUMAN MONOAMINE OXIDASE (MAO) ENZYMES

Product No.	Description
CYP155	Human Monoamine Oxidase A (MAO-A)
CYP156	Human Monoamine Oxidase B (MAO-B)
CYP208	Control Sf9 microsomes

PRODUCT DESCRIPTION:

Recombinant human MAO enzymes are supplied as microsomes, isolated from Sf9 insect cells (derived from *Spodoptera frugiperda*), following infection with recombinant baculovirus. The microsomes are supplied in 1x PBS [10 mM sodium phosphate buffer (pH 7.4), 140 mM sodium chloride] containing 20% (v/v) glycerol.

STORAGE: ≤ -80°C

MATERIALS

200 mM potassium phosphate, pH 7.4
Deionized water
Substrate solution
1x PBS containing 20% (v/v) glycerol (for enzyme dilution, if required)
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 90 mM potassium phosphate buffer, pH 7.4, although other buffers may be used.

DRUG METABOLISM

1) Thaw the insect cell microsomes and keep on ice once thawed. Mix the microsomes by gently vortexing 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	90 µl
Water	(110 – x – y) µl
Substrate / test compound	x µl
MAO	y µl

Pre-mix for 20 x 0.2 ml incubations:

200 mM potassium phosphate pH 7.4	1800 µl
Water	(2200 – xx – yy) µl
Substrate / test compound	xx µl
MAO	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).

The concentration of MAO 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 MAO 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 MAO should be increased accordingly.

3) Add the appropriate volume of pre-mix (with either the substrate or the MAO omitted, depending on which is being used to initiate the reaction) to each assay tube (1.5 ml polypropylene microtubes work well) and pre-incubate at 37°C for 5 min. The assay volume can be adjusted as required: we also use 1 ml final volume assays in 15 ml polypropylene conical tubes.
4) Initiate the reaction by adding either substrate or MAO to each tube, and incubate at 37°C (typically 5 – 30 minutes, but this depends on the MAO 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:

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North America & Asia Pacific
516-483-1196
customerservice@bioivt.com
PO Box 770, Hicksville NY 11802-0770,U.S.A.

Europe, Middle East & Africa
44 1444 707333
cseurope@bioivt.com
West Sussex RH15 9TN, U.K.

BioIVT.com