Recombinant Human Aldehyde Oxidase (AOX) enzymes

Product No.	Description
CYP150	Aldehyde Oxidase
CYP099	Control cytosol

Product Description:

Human aldehyde oxidase (AOX) recombinant enzymes are expressed in E-coli (10 mg/mL) and are supplied in 25 mM potassium phosphate (pH 7.4), containing 0.1 mM EDTA ('PE25'). Each vial of human AOX recombinant enzyme contains 2 mg cytosolic protein.

Storage: **≤ -80°C**

Materials

25 mM potassium phosphate, pH 7.4, containing 0.1 mM EDTA ('PE25': assay buffer; also used for enzyme dilution, if required)
Substrate solution (dissolve the substrate in assay buffer, if possible)
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 25 mM potassium phosphate buffer, pH 7.4, containing 0.1 mM EDTA (PE25), although other buffers may be used.

Drug Metabolism

1) Thaw the AOX 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:

PE25 buffer	(200 – x – y) µl
Substrate / test compound	x µl
AOX	y µl

Pre-mix for 20 x 0.2 ml incubations:

PE25 buffer	(4000 – xx – yy) µl
Substrate / test compound	xx µl
AOX	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 AOX 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 AOX 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 AOX should be increased accordingly.

3) Add the appropriate volume of pre-mix (with either the substrate or the AOX omitted) 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 tubes.
4) Initiate the reaction by adding either substrate or AOX to each tube, and incubate at 37°C. When using phthalazine as substrate, the reaction is only linear for 1 – 2 minutes, but longer incubation times may be possible with alternative substates/buffers.
5) Stop the reaction(s) by the addition of one of the following:
   0.125 volumes 1 M HCl (25 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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