RECOMBINANT HUMAN ALDEHYDE DEHYDROGENASE (ALDH) ENZYMES

Product No.	Description
CYP151	Aldehyde Dehydrogenase 1A1
CYP099	Control cytosol

PRODUCT DESCRIPTION:

Human aldehyde dehydrogenase (ALDH) recombinant enzymes are expressed in E-coli (10 mg/mL) and are supplied in 10 mM sodium phosphate (pH 7.4), 140 mM NaCl. Each vial of human ALDH recombinant enzyme contains 1 mg cytosolic protein.

STORAGE: ≤ -80°C

MATERIALS

50 mM HEPES, pH 7.4 (assay buffer; can also be used for enzyme dilution)
0.5 M EDTA, pH 8.0
Deionized water
Substrate solution (dissolve the substrate in assay buffer, if possible)
1 M dithiothreitol in water (prepare fresh or store in aliquots at -20°C)
10 mM NAD+ in 50 mM HEPES, pH 7.4 (prepare fresh and keep on ice until required)
1x PBS (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 50 mM HEPES buffer, pH 7.4, containing 1 mM EDTA and 2 mM dithiothreitol, although other buffers may be used.

DRUG METABOLISM

1) Thaw the ALDH and keep on ice once thawed. Mix gently just before use.
2) Prepare incubations on ice, using the guide below. Reactions are initiated by the addition of the cofactor NAD+. Multiple incubations should be prepared from a pre-mix (see below).

For a single 0.5 ml incubation:

50 mM HEPES, pH 7.4	(473 – x – y) µl
0.5 M EDTA	1 µl
1 M dithiothreitol	1 µl
Substrate / test compound	x µl
ALDH	y µl

Pre-mix for 20 x 0.5 ml incubations:

50 mM HEPES, pH 7.4	(9460 – xx – yy) µl
0.5 M EDTA, pH 8.0	20 µl
1 M dithiothreitol	20 µl
Substrate / test compound	xx µl
ALDH	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 ALDH 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 ALDH 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 ALDH should be increased accordingly.

3) Add 475 µl of pre-mix 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 25 µl of 10 mM NAD+ to each tube (final NAD+ concentration 0.5 mM), and incubate at 37°C (typically 5 – 30 minutes, but this depends on the ALDH and substrate being used). Higher final concentrations of NAD+ may result in higher activity.
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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