Recombinant Human Sulfotransferases (SULTs) enzymes

Product No.	Description
CYP100	SULT1A1*1
CYP101	SULT1A3
CYP102	SULT1B1
CYP103	SULT1E1
CYP104	SULT2A1
CYP105	SULT1A1*2
CYP106	SULT1A2
CYP107	SULT1C2
CYP108	SULT1C4
CYP099	Control cytosol

Product Description:

Human sulfotransferases (SULTs) 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 SULT recombinant enzyme contains 1 mg cytosolic protein.

Storage: **≤ -80°C**

Materials

200 mM potassium phosphate, pH 7.4
100 mM magnesium chloride
Deionized water
Substrate solution
1 M dithiothreitol in water (prepare fresh or store in aliquots at -20°C)
PAPS cofactor (3'-phosphoadenosine-5'-phosphosulfate; suggested stock solution 0.4 mM in 10 mM potassium phosphate, pH 7.4. Prepare fresh or store in aliquots at -80°C. Do not re-freeze after thawing)
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 potassium phosphate buffer, pH 7.4, containing 5 mM magnesium chloride and 10 mM dithiothreitol, although other buffers may be used.

DRUG METABOLISM

1) Thaw the SULT 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 PAPS. 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
100 mM magnesium chloride	10 µl
Water	(128 – x – y) µl
1 M dithiothreitol	2 µl
Substrate / test compound	x µl
SULT	y µl

Pre-mix for 20 x 0.2 ml incubations:

200 mM potassium phosphate pH 7.4	1000 µl
100 mM magnesium chloride	200 µl
Water	(2560 – xx – yy) µl
1 M dithiothreitol	40 µl
Substrate / test compound	xx µl
SULT	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 SULT 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 SULT 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 SULT should be increased accordingly.

3) Add 190 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 10 µl of 0.4 mM PAPS to each tube (final PAPS concentration 20 µM), and incubate at 37°C (typically 5 – 30 minutes, but this depends on the SULT and substrate being used). Higher final concentrations of PAPS 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:

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.