1.0 INTRODUCTION

Sulfotransferase enzymes catalyze the conjugation of sulfate groups onto a variety of xenobiotic and endogenous substrates that possess acceptor moieties such as hydroxyl and amine groups:

\[
\text{Sulfotransferase enzymes} \\
R-XH + \text{phosphoadenosine phosphosulfate} \rightarrow R-\text{SO}_4 + \text{phosphoadenosine} + H^+
\]

The cofactor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is required for sulfonation by these enzymes. Although sulfonation generally causes molecules to lose their “biological activity”, several documented examples indicate that the addition of sulfate can lead to the formation of active metabolites, such as minoxidil, and reactive electrophiles, such as sulfonated N-hydroxy-2-acetylaminofluorene.\(^1\,^2\) Several sulfotransferase enzymes with different biochemical properties have been characterized in animal and human tissue. Two general classes of these enzymes exist in tissue fractions: the cytosolic enzymes that are important in drug metabolism, and the membrane-bound enzymes that are involved in the sulfonation of glycosaminoglycans and glycoproteins.\(^3\) The human cytosolic sulfotransferase isozymes function as homodimers of 32-35 kD subunits. These enzymes are products of multiple genes, of which five are currently known (SULT1A1, SULT1A2, SULT1A3, SULT1E and SULT2A1).

It is expected that other genes encoding for sulfotransferases will be identified. The nomenclature of the different genes and their mRNA and protein products has recently been revised so that “SULT” is the accepted superfamily abbreviation.\(^4\) Allelic variants of sulfotransferase enzymes do exist, and studying their frequency and functional role in drug disposition is a very active area of research. This important class of enzymes belongs to the Phase II group of drug metabolizing enzymes, which also includes the glycosyltransferases, epoxide hydrolases, and N-acetyl transferases.

PanVera provides cytosolic extracts prepared from insect cells infected with a baculovirus strain that contains cDNA that encodes for a human sulfotransferase. Several important classes of human sulfotransferases are now available including: SULT1A1*2, SULT1A2*1, SULT1A3, SULT1E and SULT2A1.

2.0 SAFETY PRECAUTIONS

Normal precautions exercised in handling laboratory reagents should be followed. Refer to the Material Safety Data Sheet(s) for any updated risk, hazard or safety information. The reagents supplied are not considered hazardous according to 29 CFR 1910.1200. The chemical, physical, and toxicological properties of these products may not, as yet, have been thoroughly investigated. We recommend that you use gloves, lab coats and eye protection when working with any chemical reagents.

3.0 PROCEDURE

Like the cytochrome P450 enzymes and UDP-glycosyltransferases, sulfotransferases show some degree of overlapping substrate specificity. Therefore, it is important to perform experiments under linear conditions and define the kinetic parameters for substrate conjugation before making conclusions about isoform specificity. These enzymes also show significant substrate inhibition\(^5\), and we recommend trying a variety of substrate concentrations in your experiments. Sulfotransferase isoforms have been characterized in tissue fractions by measuring the sulfate conjugation of various acceptor substrates attributed to them. At PanVera, we use the substrates shown below to characterize the specific activities and properties of recombinant sulfotransferase enzymes expressed in insect-cell cytosolic preparations.

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Substrate</th>
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<tbody>
<tr>
<td>SULT1A1*2</td>
<td>p-nitrophenol</td>
</tr>
<tr>
<td>SULT1A2*1</td>
<td>nitrophenol</td>
</tr>
<tr>
<td>SULT1A3</td>
<td>dopamine</td>
</tr>
<tr>
<td>SULT1E</td>
<td>estrone</td>
</tr>
<tr>
<td>SULT2A1</td>
<td>dehydroepiandrosterone</td>
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</tbody>
</table>
3.1 Reagents

**Dilution Buffer**: 5 mM potassium phosphate (pH 6.5), containing 1.5 mg/mL BSA and 10 mM dithiothreitol (DTT). Prepare each week from stock solutions of mono- and di-basic salts.

**Cocktail Buffer** (for one reaction only): 25 µL of 50 mM potassium phosphate (pH 6.5), 25 µL of 7.4 mg/mL dithiothreitol, and 1.28 µM [35S]PAPS (NEN, NEG-010). Please note that some laboratories prepare this buffer at different pH values, depending on the sulfotransferase enzyme activity being measured. This can lead to differences in the kinetic parameters that are obtained.

**Stop Mixture**: a 1:1 (vol/vol) mixture of 0.1 M Ba(OH)₂ and 0.1 M barium acetate made fresh each day

0.1M ZnSO₄
0.1M Ba(OH)₂
50 mM MgCl₂ for SULT1E assays
10 mM MgCl₂ for SULT2A1 assays
32 mM pargyline for SULT1A3 assays or assays involving monoamines

**Substrates**: 64 µM p-nitrophenol (for SULT1A1*2)
640 µM nitrophenol (for SULT1A2*1)
1.92 mM dopamine (for SULT1A3)
32 µM estrone dissolved in DMSO (for SULT1E)
160 µM dehydroepiandrosterone (DHEA) dissolved in DMSO (for SULT2A1)

3.2 Procedure For a Single Reaction:

To simplify pipetting, prepare enough cocktail buffer for all reactions being performed. The volume of PAPS is generally negligible, and is ignored when starting the reaction. The final concentration of [35S]PAPS in the reaction is approximately 0.4 µM.

Dilute the cytosolic sample appropriately in ice-cold dilution buffer and keep chilled. Cytosolic dilutions that, in our hands, produce soluble counts in the 12-40,000 cpm range are provided on the certificate of analysis.

1. Add 10 µL of substrate/additive to a 1.5 mL microcentrifuge tube and place on ice (see section 3.4 below).
2. Add 100 µL of diluted cytosol.
3. Start the reaction by adding 50 µL of cocktail buffer.
4. Incubate at 37°C for 20 minutes.
5. Stop the reaction by adding 100 µL of the stop mixture, and vortex to mix. Add 50 µL of 0.1 M ZnSO₄ and vortex again. A precipitate will form.
6. Centrifuge for 3 minutes at maximum speed in a microcentrifuge.
7. Add 50 µL of 0.1 M Ba(OH)₂ to the tube and vortex to mix. Add 50 µL of 0.1 M ZnSO₄ and vortex again.
8. Centrifuge for 10 minutes at maximum speed in a microcentrifuge, and aliquot 300 µL of the supernatant into a scintillation vial. Add 5 mL of scintillation fluid and count.
3.3 Notes

We recommend performing duplicate or triplicate reactions for each assay point. Some laboratories add an additional 100 μL of ddH₂O in step 7 of the procedure and then remove a larger volume for scintillation counting.

Control reactions are very important and must be run with every experiment. These reactions should include:

i) A control without substrate. This control indicates if there is an endogenous acceptor present in the assay (either in a buffer or cytosol component). This is a very important control, as there are endogenous substrates present in the cytosolic extracts. If any sulfotransferase activity is observed in this control, its value should be subtracted from the activity seen in the presence of substrate. Sulfotransferases can sulfonate certain buffer components.

ii) Assays using control cytosolic extracts. Endogenous insect sulfotransferase activity is present in the cytosolic extracts provided. This activity appears to be sensitive to MgCl₂ concentration (see Figures 1 and 2). A mock-infected, control cytosolic extract is available from PanVera (Part # P2469).

3.4 Substrates and Additives for Specific Sulfotransferase Extracts

Note: The substrate and other additions should not exceed 10 μL.

SULT1A1*2: p-nitrophenol, final reaction concentration=4 μM (10 μL of a 64 μM stock solution per reaction)

SULT1A2*1: nitrophenol, final reaction concentration=20 μM (5 μL of a 640 mM stock solution per reaction)

SULT1A3: dopamine, final reaction concentration=60 μM (5 μL of a 1.92 mM stock solution per reaction). In addition, 5 μL of 32 mM pargyline, a monoamine oxidase inhibitor, is used per reaction.

SULT1E: estrone, final reaction concentration=1 μM (5 μL of a 32 μM stock solution per reaction). In addition, 5 μL of 50 mM MgCl₂ is used per reaction.

SULT2A1: dehydroepiandosterone, final reaction concentration=5 μM (5 μL of a 160 μM stock solution per reaction). In addition, 5 μL of 10 mM MgCl₂ is used per reaction.

The specific activity of the extract toward a substrate is calculated by assuming that the soluble cpm is due to sulfate conjugated substrate.

![Image of Fig. 1](image_url)

**Fig. 1. Magnesium chloride sensitivity of endogenous sulfotransferase enzyme activity in control extracts.**

Sulfotransferase enzyme activity was measured using a precipitation assay that removes [³⁵S]PAPS as described in Sect. 3.2. Briefly, the cytosolic extract was diluted to 16,000 ng/mL in pre-chilled dilution buffer. To start the reaction, a 100 μL aliquot of the diluted cytosolic extract was mixed with 5 μL of 10 mM MgCl₂, and 50 μL of cocktail buffer. The mixture was incubated at 37°C for 20 minutes, and the reaction was stopped by adding 50 μL of stop mixture. Supernatant was collected and counted.
Fig. 2 Presence of an endogenous sulfotransferase enzyme activity in control extracts in the presence of different substrates/additives. Assays were performed as described in the legend to Fig. 1. The substrate concentrations used are indicated in the figure and represent typical assay conditions used to measure activity of the recombinant enzymes.

4.0 REFERENCES