PolyCYPs Case Studies

Scale-up of PolyCYPs reactions

Once a target metabolite or oxidised derivative has been synthesised by one or more PolyCYPs enzymes in the screening kit, a scale-up reaction with the best performing isoform is performed in order to access mg amounts of material for MetID and biological testing. The quickest and most cost effective route for generating low mg amount of product is through the use of scale-up vials. Higher amounts of product can be generated using either a recombinant E.coli cell paste or through fermentation of a recombinant Streptomyces clone expressing the isoform responsible for the biotransformation.

In this client case study, milligram amounts of specific oxidised products of two compounds were required. Both screening and scale-up reactions were conducted by Hypha for the client and the extract provided to the client for purification. Scale-up reactions were performed using 500 ml of disrupted E.coli cell pellet of PolyCYPs 196 and 152, dosed at 100 mg/L substrate formulated with a cyclodextrin. Both reactions resulted in >50% turnover of the substrate. PolyCYPs reactions were also compared with biotransformations achieved from exposing the substrate to a panel of 16 wild type microbes, proven to be talented at metabolising a wide variety of drug compounds.

For compound 1, several monohydroxylated metabolites were formed with the cleanest biotransformation to the target product achieved by PolyCYP 196. For compound 2, a single monohydroxylated target product was produced by PolyCYPs enzymes only, with PolyCYP 152 being the best.

**PolyCYPs scale-up options**

<table>
<thead>
<tr>
<th>Scale-up vials</th>
<th>Enzyme Prep (Hypha)</th>
<th>Fermentation (Hypha)</th>
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<tbody>
<tr>
<td>1 vial per 1 mg substrate</td>
<td>Crude enzyme prep</td>
<td>Whole cell scale-up using streptomycete clone (or originating wild type microbe)</td>
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<td>For 100s µgs to low mg requirements</td>
<td>Takes ~2 wks for provision of reaction extract to client</td>
<td>Yield could be different or altered side products</td>
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<tr>
<td>Immediate availability</td>
<td>Optional reaction, purification and structure elucidation at Hypha</td>
<td>Takes 6-7 wks from receipt of substrate</td>
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</table>

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**Compound 1**
- Biotransformed by multiple microbes and PolyCYPs
- Target product produced best by PolyCYP 196

**Compound 2**
- Biotransformed to target oxidised products by PolyCYPs only (152 best)
- Other minor product also of interest
- Glucuronidation and other biotransformations dominated in microbial whole cell incubations

Questions? Email us at enquiries@hyphadiscovery.co.uk