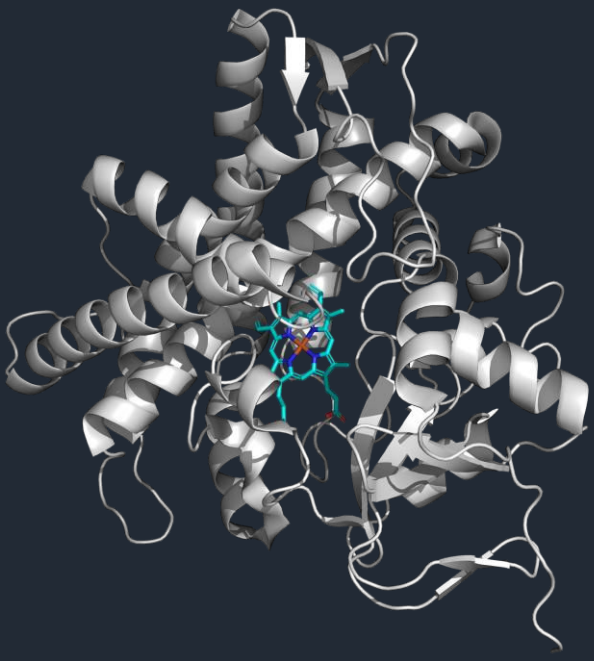
Manual

Human CYP Screening Kit
Cytochrome P450 enzymes



for

Drug discovery, Toxicity screening,
Metabolite analysis & Biocatalysis

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ALL ABOUT ENZYMES

Dear Scientist

Thank you for purchasing our Human CYP Screening Kit.

The Human CYP Screening Kit may only be used according to these instructions and by qualified personnel.

We always strive to further improve our products, so please feel free to contact us at enzymes@aminoverse.com in case you have any feedback or open questions.

We wish you a successful screening!

The Aminoverse Team

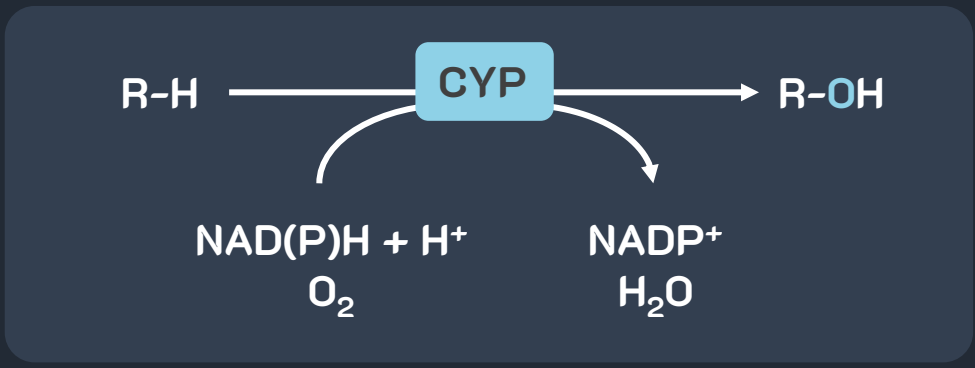
Content

1 Introduction

Cytochrome P450 enzymes (**CYP**, EC 1.14) are a large superfamily of enzymes, that catalyze mixed-function oxidation reactions of diverse substrates, such as xenobiotics (drugs, toxins), steroids, fatty acids, and eicosanoids. Key reactions include C-hydroxylation, N-, O-, and S-dealkylation, epoxidation, sulfoxidation, and reduction of azo/nitro groups.
CYPs are heme-containing enzymes and require NAD(P)H as cofactor for electron regeneration for activation of molecular oxygen and oxidation of substrates.

CYPs are proven candidates to catalyze challenging chemical reactions, e.g., for

- Drug discovery
- Toxicity screening
- Metabolite analysis
- Biocatalysis



Free of third-party IP

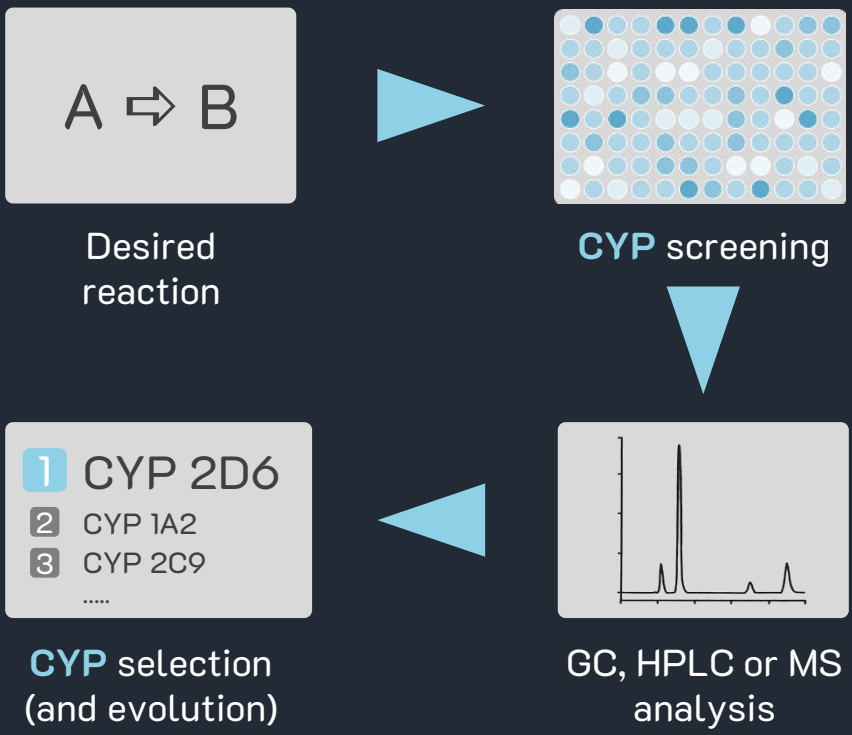
Available up to kg scale

2 The Human CYP Screening Kit

The **CYP** Screening Kit contains Human **CYP** enzymes, recombinantly produced in *Pichia pastoris* for whole cell catalysis.

The flexible selection of volumes and format allows parallel screening of the entire **CYP** Screening Kit on different substrates, followed by your GC, HPLC or MS analysis of choice.

Any **CYP** from the **CYP** Screening Kit yielding the desired product can be engineered towards even greater performance and is available up to kg scale.

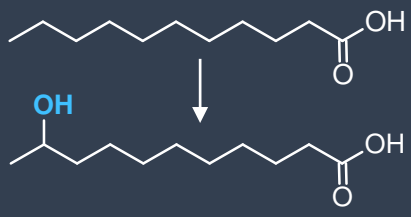


3 Example reactions

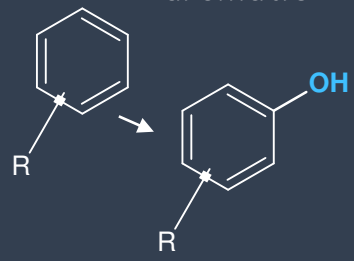
Examples for oxidation reactions, catalyzed by **CYPs**

Hydroxylation

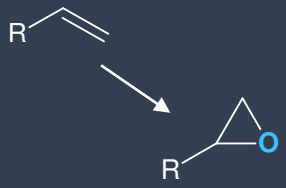
aliphatic



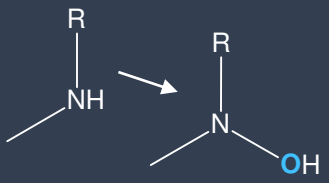
aromatic



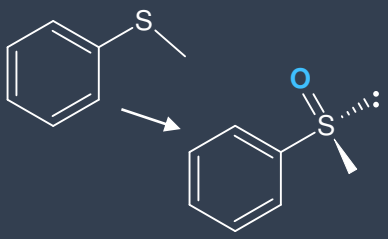
Epoxidation



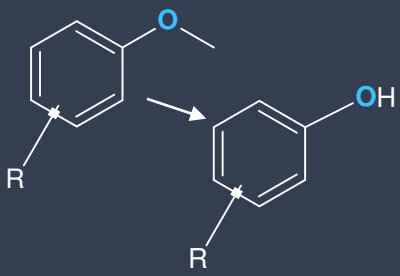
N-hydroxylation



Sulfoxidation



Dealkylation



[1]

[1] https://doi.org/10.1016/j.redox.2014.11.008

4 Components and storage

Human **CYP** Screening Kit

- Contains 8x 1 g lyophilized *Pichia* whole cell catalyst + 1 negative control

8 human **CYPs**

- 1A1
- 1A2
- 2C8
- 2C9
- 2C19
- 2E1
- 2D6
- 3A4

- Recombinantly produced in *Komagataella phaffii (Pichia pastoris)*
- Lyophilized in 100 mM KP_i buffer, containing 250 mM sucrose

- Store at -20 °C or preferably at -80 °C
- Use directly after resuspension
- Caution: Avoid repeated freeze-thaw cycles of the **CYP** whole cell catalyst

Reactions possible with 1 **CYP** Screening Kit:

- If you follow the recommended reaction protocol, one kit provides for the performance of 80x 100 µL or 32x 250 µL reactions per **CYP** enzyme
- The negative control (*Pichia pastoris* empty strain) can be used to rule out any non-CYP-catalyzed background reaction

5 Required reagents and equipment

- Reaction buffer: 100 mM phosphate, pH 7.4
- Substrate stock solution, 20x concentrated
    - e.g., 100 mM stock solution for 5 mM under reaction conditions
- Stop solution: Methanol/Acetonitrile (1:1)
- Solvent-resistant reaction vessels, such as *e.g.*:
    - (polypropylene) microtiter plates (MTP)
    - (polypropylene) reaction tubes
    - Glass vials
- Gas-permeable seal foil (e.g., Thermo Scientific #AB-0718)
- Micro pipette
- Shaking incubator
- *Optional:* ***NAD(P)H:*** The whole cell catalyst itself contains NAD(P)H. Increased NAD(P)H availability can be beneficial and can be provided by addition of 50 μM $NADP^+$ (reaction conditions)
- *Optional:* ***NAD(P)H regeneration system*** to maintain a constant supply of the NADPH cofactor, composed of e.g., (reaction conditions)
    - 0.15 U glucose dehydrogenase
    - 50 mM sodium citrate
    - 10 mM magnesium chloride
    - 10 mM glucose

6 Protocol

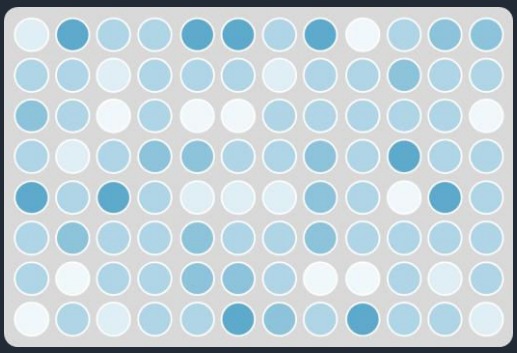
- Reaction volumes, reagent concentrations, and incubation times may be adapted to the desired reaction and sensitivity of detection the method.
- Reaction solutions can be analyzed by GC, HPLC or MS. Depending on the chosen analysis method, the extraction procedure may vary.
- Important: **CYP** preparations are living GMOs. Inactivate as per BSL-1 regulations before disposal.

1. Prepare a **CYP** solution by resuspending the **CYP** whole cell catalyst in Reaction buffer to a concentration of 125 mg/mL
2. Add **CYP** solution to a suitable reaction vessel, e.g. microtiter plate or tube
3. Start the reaction by addition of substrate stock solution and cover the plate with a gas-permeable seal foil
4. Incubate at 28 °C for 16 h at 120 rpm
5. Stop the reaction by addition of 1:1 (v/v) Stop solution
6. Centrifuge the vessel at max. speed, 4 °C for 10 min to pellet the cells
7. Use the supernatant to continue with your preferred analytical method to determine substrate decrease/product increase

7 Pipetting scheme

	Concentration		Volume [µL]	
Compound	Stock	Reaction	MTP	Tube
CYP solution in reaction buffer	125 mg/mL	119 mg/mL	95	225
Substrate solution	100 mM	5 mM	5	25
Reaction volume			**100**	**250**
Stop solution			100	250
Final volume			200	500

- Reactions can be performed *e.g.*, in microtiter plates or reaction tubes



8 Adapting the protocol

Reaction volume

- Can be adjusted; *e.g.*, scaled up to 1000 µL

Reaction time

- Conversion rates of substrates can vary and reaction time can be varied according to specific needs. Shorter incubation time may be sufficient for complete conversion. Extending reaction time up to 24 h can lead to higher conversion ratio.

Substrate stock solution & CYP concentration

- Catalytic activity (affinity) with different substrates may vary. Substrate concentration can be adjusted to specific needs and according to solubility.
- For verification of **CYP** activity, e.g., using fluorescent substrates, lower substrate.
- Amount of **CYP** whole cell catalyst in reaction may be tuned according to specific needs. E.g., increasing the **CYP** catalyst concentration by dissolution of lyophilizate at higher concentration.

8 Adapting the protocol

Increased NAD(P)H availability

- The whole cell catalyst itself contains NAD(P)H. Increased NAD(P)H availability can be beneficial and can be provided by addition of 50 μM $NADP^+$ or a NAD(P)H regeneration system (page 8).

Reaction stop

- If quenching with Methanol/Acetonitrile interferes with reaction product analysis, the reaction can be stopped by centrifugation of the reaction to separate the whole cell catalyst from the reaction product in supernatant.

Feel free to contact us in case of questions or for tuning of reaction conditions enzymes@aminoverse.com

Application examples:

- Geier et al., *Biotechnol. J.* **2012**, *7*, 1346–1358.
- Migglautsch et al., *Tetrahedron* **2018**, *74*, 6199–6204.
- Rinnofner et al., *Biocatal. Agric. Biotechnol.* **2019**, *17*, 525–528.

9 Miscellaneous

The **CYP** Screening Kit is for *in vitro* research use only, not for diagnostic or therapeutic applications and has to be handled by qualified personnel.

CYP preparations are living GMOs. Inactivate as per BSL-1 regulations before disposal. Recultivation is strictly forbidden, and no attempts at amplification, propagation, or replication are allowed.

The purchase of this product only authorizes the buyer to application of the **CYP** Screening Kit for internal research. Consult Aminoverse's Terms and Conditions for more information.

Reference information:
In publications, please refer to the **CYPs** of this Screening Kit by their *ID* as "Aminoverse *AVCYP0800*".

About the CYP Screening Kit

The **CYP** Screening Kit was created to meet the increasing demand for efficient biocatalysts for oxyfunctionalization reactions.

Made possible thanks to our partner

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AUSTRIA
bisy.at

AMINOVERSE
ALL ABOUT ENZYMES

Aminoverse solves enzyme challenges.
Founded in 2020, Aminoverse offers innovative enzyme products and services:

- Enzyme products and kits
  Ready-to-use enzymes for proof-of-concept studies up to commercial scale, *e.g.,* the KGO Enzyme Panel, the UPO Enzyme Panel, or the Lipase and analyte detection kits, *e.g.,* the Phosfinity series.

- CRO services
  Discovery of enzymes, enzyme feasibility studies, engineering of enzymes by Directed Evolution, *in silico design* and machine learning.

You have questions regarding this manual or about the application of the Human **CYP** Screening Kit?

We look forward to hearing from you.

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