Partnering to Advance Human Health

Hyperactivated Hydrolase Enzyme Screening Kit

HAHESK-1000 (50 mg)

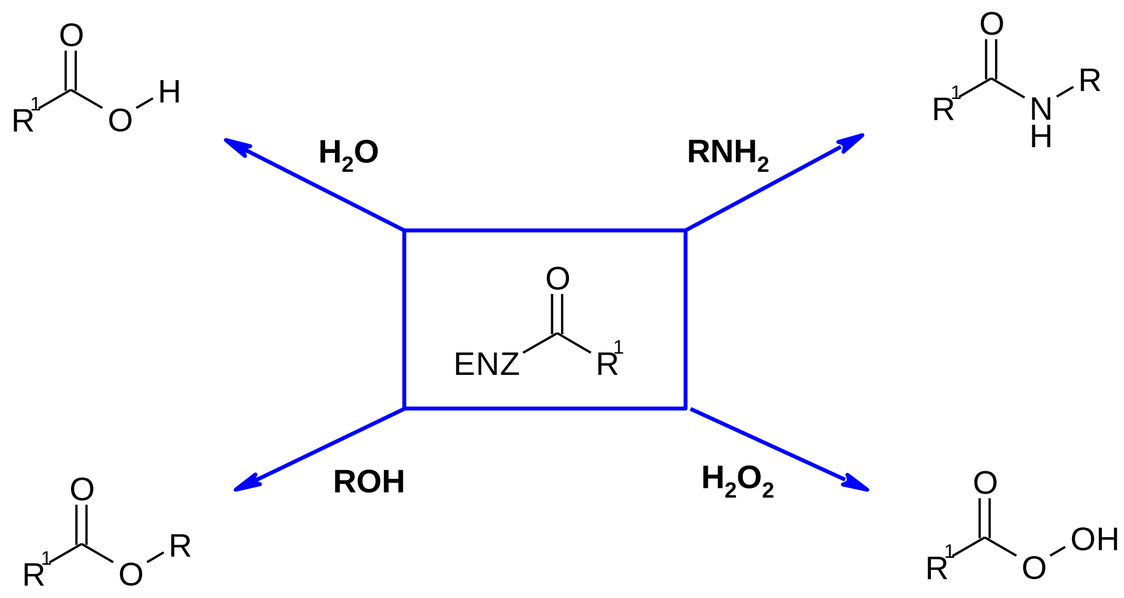
selectAZyme™

Applications

Selective hydrolysis in aqueous media, selective acylation in non-aqueous media, resolution of secondary alcohols, amines and thiols, formation of peptides.

Kit description

The kit contains 10 commercial hyperactivated hydrolase biocatalysts, as well as pre-prepared phosphate buffer for hydrolysis and a selection of acyl donors for selective acylation.



Enzymes included in this kit

HAH-001	HAH-006
HAH-002	HAH-007
HAH-003	HAH-008
HAH-004	HAH-009
HAH-005	HAH-010

Contents

Hydrolase enzymes	10 vials (50 mg each)
Vinyl acetate	1 bottle (1 mL)
Vinyl butyrate	1 bottle (1 mL)
Succinic anhydride	1 bottle (1 g)
Ethyl acetate	1 bottle (1 mL)
Ethyl butyrate	1 bottle (1 mL)
0.1M KH_2PO_4 buffer (pH 7.0)	1 bottle (200 mL)

Storage: The hyperactivated hydrolase enzyme screening kit should be stored in a refrigerator at <4 °C to preserve activity.

Bioresolution of alcohols

There are three main strategies for resolving primary, secondary and tertiary alcohols: transesterification of the alcohol to an ester, hydrolysis of an ester to the alcohol, or alcoholysis of an ester to an alcohol. Screening protocols for each strategy are shown below.

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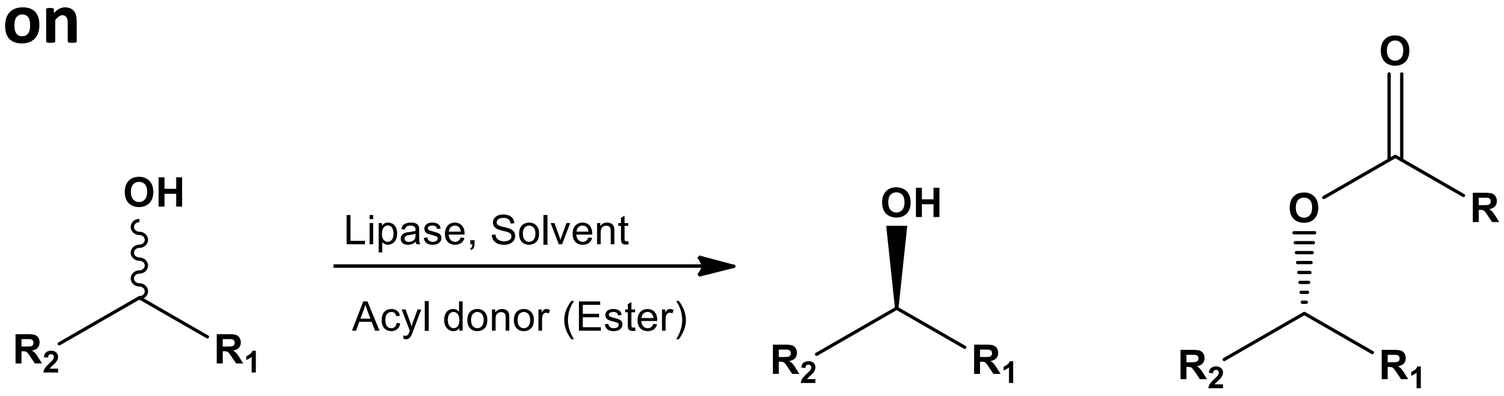
Transesterification



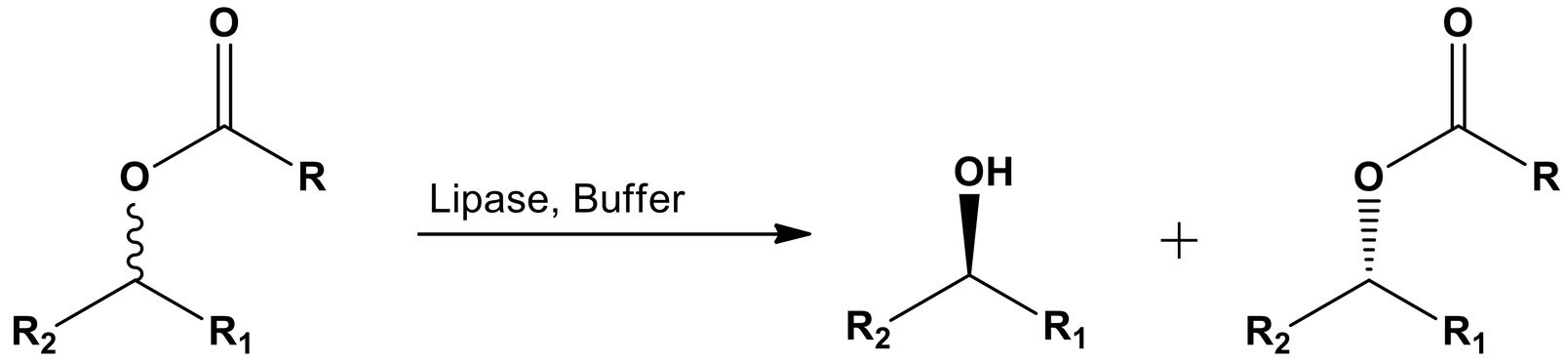
1.Dissolve the substrate to a concentration of 20 mg/mL in a solvent such as MTBE together with an acyl donor such as vinyl acetate (1 eq) and dispense 1 mL (20 mg) into small reaction vessels (such as HPLC vials). If the substrate is insoluble in this solvent, others such as n-heptane, Et_2O, THF, PhMe or MeCN can be tried.

2. Add enzyme to each reaction vessel (~5-10 mg – i.e. a spatula tip).

3. Close the vessels and agitate the suspension at ambient temperature.

4. After 12-16 hours, take a sample and assay for conversion, either by TLC or HPLC. If suitable conversion has occurred, a chiral assay may be performed to determine the selectivity of the reaction. If further conversion is required, continue the reaction and assay again after 24 hours.

Aqueous hydrolysis of esters



These bioresolutions are performed in neutral phosphate buffer, in which the enzyme is dissolved. The exact composition of the aqueous system depends on the substrate physical form. If the ester is a liquid, the biotransformation may simply be carried out as a biphasic mixture of buffer and ester. If the ester is a solid, it may be possible to perform the reaction with the substrate as a finely ground powder, but usually it is more convenient to add a co-solvent to solubilise the ester. This may be a water miscible solvent creating a single phase system, or an immiscible solvent creating a biphasic system. Examples of solvents that can be tried are given below. At the screening stage these are used at around 20% v/v.

This protocol assumes a relatively water-insoluble solid ester as the substrate:

1.Dissolve substrate to 80 mg/ml in a solvent such as MTBE and dispense 250 μL (20 mg) into small reaction vessels (such as HPLC vials).

2. Add phosphate buffer (1 mL) to each vessel.

3. Add enzyme (~5-10 mg) to each vessel, close, and agitate at ambient temperature by shaking, so that the suspension is well mixed.

4. After 12-16 hours, take a sample and assay for conversion, either by TLC or HPLC. If suitable conversion has occurred, a chiral assay may be performed to determine the selectivity of the reaction. If further conversion is required, continue the reaction and assay again after 24 hours.

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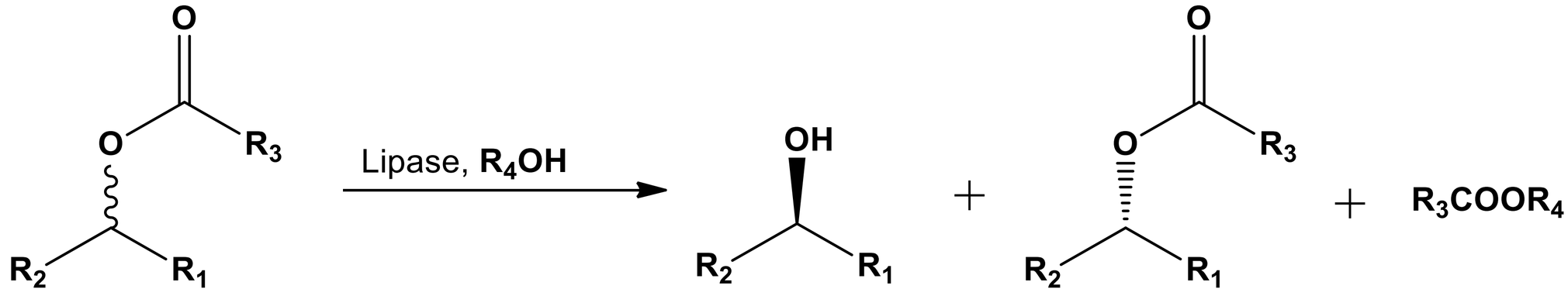
Alcoholysis of esters

Sometimes it is convenient to resolve esters by performing a non-aqueous alcoholysis of the racemate. Only simple alcohols such as MeOH, EtOH, *n*-PrOH, *i*-PrOH or *n*-BuOH are used, and they are usually used as the solvent for the reaction (except MeOH, which tends to denature enzymes too much).



1. Dissolve the ester to a concentration of 10 mg/mL in a suitable alcohol such as *n*-BuOH and dispense 1 mL (10 mg)) into small reaction vessels (such as HPLC vials).

2. Add enzyme to each reaction vessel (~5-10 mg), close, and agitate at ambient temperature by shaking, so that the suspension is well mixed.

3. After 24 hours, take a sample and assay for conversion, either by TLC or HPLC. If suitable conversion has occurred, a chiral assay may be performed to determine the selectivity of the reaction. If further conversion is required, continue the reaction and assay again after 72 hours.

Bioresolution of carboxylic acids

Aqueous hydrolysis of esters

Chiral carboxylic acids are most frequently resolved by hydrolysis of the ester, and screening can be performed exactly as previously described for hydrolysis of the esters of chiral alcohols. As for chiral alcohols, the solvent system used for the ester hydrolysis screen may be mono- or biphasic, and a biphasic MTBE/buffer mixture is suitable for an initial screen.

Non-aqueous esterification of carboxylic acids

Occasionally the screening may be carried out directly on the carboxylic acid, where the enzyme catalyses the formation of an ester in the presence of a suitable alcohol. This is usually best completed using a short chain alcohol as the solvent (EtOH, *n*-PrOH, *i*-PrOH, *n*-BuOH, etc.) to help drive the reaction to completion, otherwise the catalytic system tends towards an equilibrium, which in a bioresolution reduces the enantiomeric excess of substrates and product. It is beneficial to remove the water formed during the esterification so as to prevent the unwanted reverse reaction. Thus, it is recommended that molecular sieves are added to such reactions.

1. Dissolve the carboxylic acid to a concentration of 10 mg/mL in a suitable alcohol such as *n*-BuOH and dispense 1 mL (10 mg) into small reaction vessels (such as HPLC vials).

2. Add enzyme to each reaction vessel (~5-10 mg), followed by molecular sieves (~10-20 mg). Close reaction vessels and agitate at ambient temperature by shaking, so that the suspension is well mixed.

3. After 24 hours, take a sample and assay for conversion, either by TLC or HPLC. If suitable conversion has occurred, a chiral assay may be performed to determine the selectivity of the reaction. If further conversion is required, continue the reaction and assay again after 72 hours.

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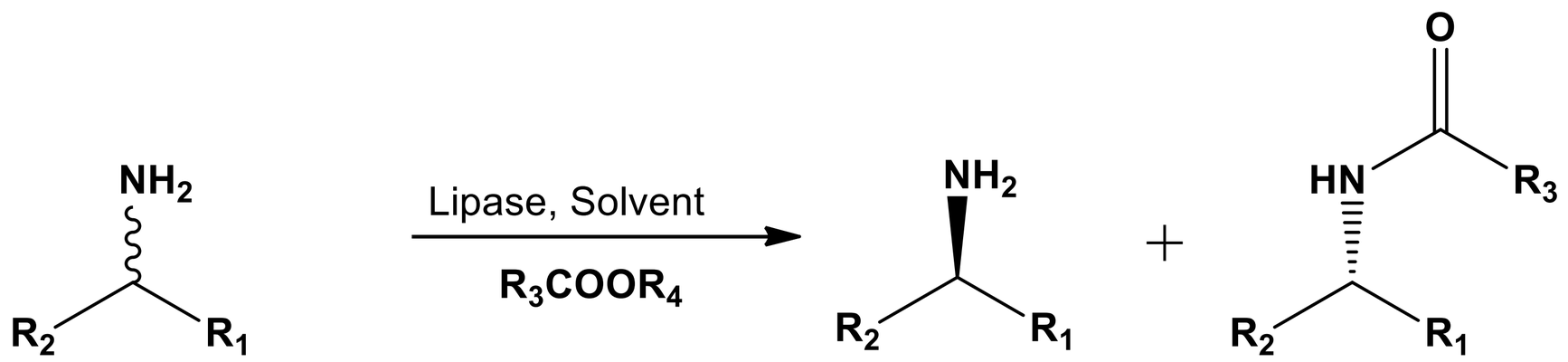
Bioresolution of thiols

Thiols may be readily resolved by enzymes. Experimentally, screening for bioresolution of thiols or thioesters can be done in the same way as for alcohols.

Bioresolution of amines

Amines are readily resolved by enzyme-catalysed acylation, providing a valuable alternative approach to crystallisation methods. The amine replaces the hydroxyl as the nucleophile, and simple esters may be used as both solvent and acylating agent. Vinyl esters can also be used, but there may be issues of reaction of the by-product acetaldehyde with the amine.



Both primary and secondary amines have been resolved by this approach. Typical acyl donors include ethyl butyrate, ethyl acetate (or other similar esters) and ethyl chloroacetate.

1. Dissolve the amine to a concentration of 20 mg/mL in ethyl butyrate and dispense 1.5 mL (30 mg) into small reaction vessels (such as HPLC vials).

2. Add enzyme to each reaction vessel (~20 mg), close, and agitate at ambient temperature by shaking.

3. Sample after 24 hours and assay by GC or HPLC.

4. The best handful of results should then be re-screened with a small number of acylating agents and solvents.

Enantiomeric ratios

An important calculation used in bioresolution is the enantiomeric ratio, or E value, which gives a measure of the enantioselectivity of a bioresolution. (For a full explanation of the kinetics see Chen, J. Am. Chem. Soc., 1982, 104, 7294-7299).

The E value is formally defined as the ratio of the k_{cat}/K_m for each of the enantiomers (k_{cat} is the turnover number, K_m the Michaelis constant, and k_{cat}/K_m the specificity constant). The E value allows a judgement of the efficiency of the bioresolution to be made at any point in the reaction, in a situation where the enantiomeric excess of both the product and substrate, and the conversion, are constantly changing throughout the biotransformation as the enzyme converts the substrate into product, with varying rates for each enantiomer.

In practical terms, the higher the E value, the more selective the bioresolution is, giving a higher yield of product at higher ee. An E value of 200 is excellent, and little optimisation will be needed. An E value of 30-50 is good and will probably suffice given a further purification of the product, for example by crystallisation. An E value of 10-20 will probably need improving by further development. An E value of 1 represents an unselective bioresolution. These figures are only a guide, and the E value needed for a viable process varies depending on the economics and product quality required.

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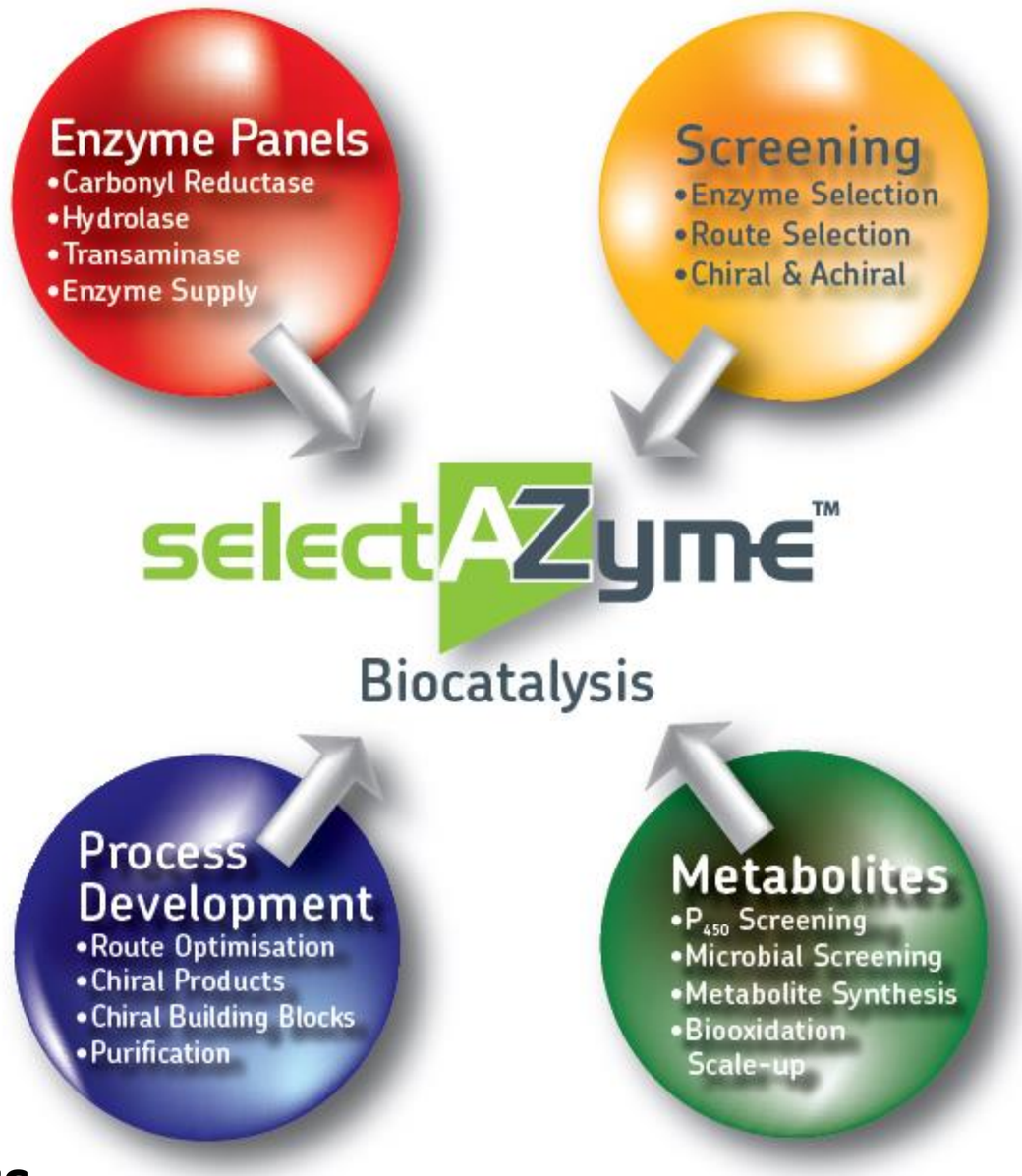
selectAZyme Offerings

- An ever-expanding biocatalysis team including molecular and microbiologists, enzymologists, bioinformaticians, organic chemists and analysts, all equipped with state-of-the art facilities.
- Expertise in gene identification, expression, fermentation and enzyme production, followed by the efficient use of enzymes to produce complex chiral APIs.
- Enzyme evolution based on computational re-design, semi-rational and random mutagenesis approaches, allowing access to bespoke biocatalysts with enhanced activity, selectivity and process robustness.
- Fully integrated biocatalyst development through screening, (chemo-) enzymatic route definition, process development and scale up (pilot plant facilities available).
- Rapid implementation of enzymatic steps in complex, multi-stage syntheses, leading to significant improvements in production yields and timelines.
- A simple business model that avoids IP issues.



The selectAZyme Range of Enzyme Screening Kits

Our selectAZmye kits include a detailed user guide and come with all buffers, cofactors, recycling systems and reagents necessary to perform screens using standard laboratory equipment.

Carbonyl Reductase (CRED) biocatalysts

96 CRED biocatalysts for the production of chiral alcohols and/or use in cofactor recycling schemes

Aldehyde Reductase (ARED) biocatalysts

16 ARED biocatalysts

Hydrolase biocatalysts

48 commercially available hydrolases for selective acylation of alcohols and amines.

Nitrilase and Nitrile Hydratase (NHase) biocatalysts

9 NHases and 15 nitrilases

Transaminase (TAm) biocatalysts

96 TAms for the prodcution of chiral amines from pro-chiral ketones.

Ene Reductase (ERED) biocatalysts

143 ERED biocatalysts for asymmetric reduction of activated alkenes

P450 Monooxygenase biocatalysts

96 P450 monooxygenase biocatalysts for a huge range of highly selective oxidations

Want Almac to do the screening for you?

- Our experienced biocatalysis team can screen all of our enzymes against your target substrate(s) and simply provide the results.
- Flexible options for subsequent enzyme supply, evolution services, process development and scale up as required.

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