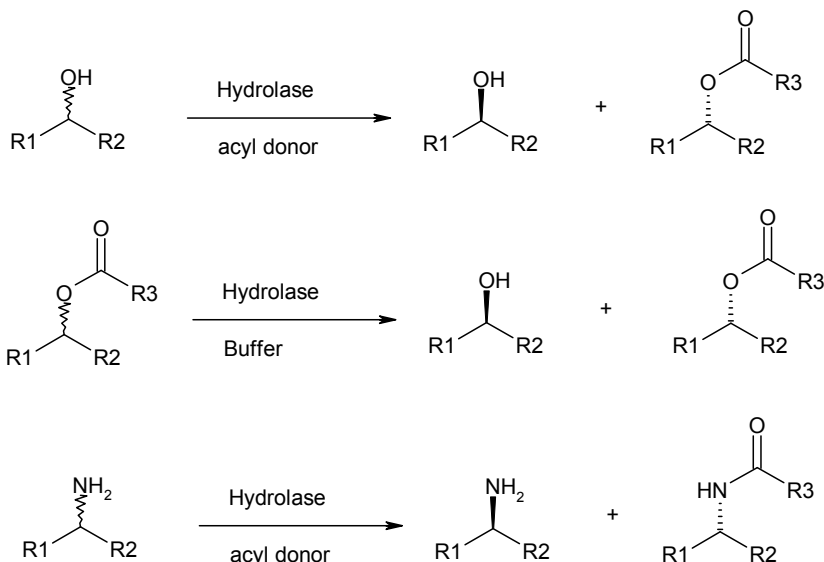


Hydrolase

Enzyme Screening Kit: HESK-4600

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 46 commercial hydrolase biocatalysts, as well as pre-prepared phosphate buffer for hydrolysis and a selection of acyl donors for selective acylation.

Contents:

Hydrolase enzymes	46 vials lyophilised powder (500 mg each)
Vinyl acetate	1 bottle (5 mL)
Vinyl butyrate	1 bottle (5 mL)
Succinic anhydride	1 bottle (5 mL)
Ethyl acetate	1 bottle (5 mL)
Ethyl butyrate	1 bottle (5 mL)
0.1M KH ₂ PO ₄ buffer (pH 7.0)	2 bottles (200 mL each)

A sufficient supply of all contents has been provided for 3 screens with each enzyme. Additional components are available for purchase from Almac.

Storage:

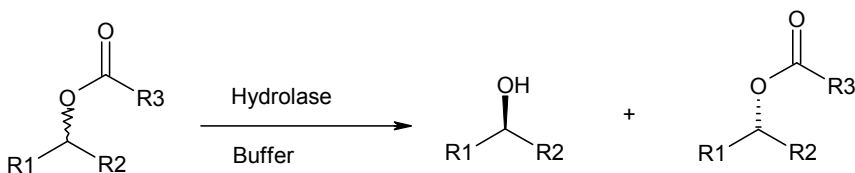
The Hydrolase enzyme screening kit should be stored in a refrigerator at <4 °C to preserve activity.

Enzyme List:

CODE	Enzyme Type
AH-01	Lipase A from Alcaligenes sp.
AH-02	Lipase B from Alcaligenes sp.
AH-03	Lipase C from Alcaligenes sp.
AH-04	Lipase from Pseudomonas stutzeri
AH-05	Lipase from Pseudomonas cepacia
AH-06	Lipase A from Candida rugosa
AH-07	Lipase D from Alcaligenes sp.
AH-08	Lipase E from Alcaligenes sp.
AH-09	Lipase B from Candida rugosa
AH-10	Lipase F from Alcaligenes sp.
AH-11	Lipase from fungal source
AH-12	Protease A from Bacillus subtilis
AH-13	Phytase
AH-14	Alkaline protease A
AH-15	Alkaline lipase A
AH-16	Lipase from Bromeliaceae sp.
AH-17	Lipase from Carica papaya
AH-18	Neutral protease A
AH-19	Alkaline protease B
AH-20	Acidic protease A
AH-21	Protease A from Aspergillus oryzae
AH-22	Protease B from Bacillus subtilis
AH-23	Acylase from Aspergillus sp.

CODE	Enzyme Type
AH-24	Lipase B from Candida rugosa
AH-25	Lipase from Rhizopus niveus
AH-26	Protease from Bacillus stearothermophilus
AH-27	Lipase from Aspergillus niger
AH-28	Lipase from Penicillium roquefort
AH-29	Protease from Aspergillus niger
AH-30	Lipase from Aspergillus oryzae
AH-31	Protease from Aspergillus melleus
AH-32	Lipase from Penicillium camembertii
AH-33	Protease C from Bacillus subtilis
AH-34	Protease B from Aspergillus oryzae
AH-35	Lipase from Pseudomonas fluorescens
AH-36	Lipase A from Burkholderia cepacia
AH-37	Lipase B from Burkholderia cepacia
AH-38	Lipase A from Rhizomucor miehei
AH-39	Lipase from Candida antarctica
AH-40	Lipase from Thermomyces lanuginosus
AH-41	Protease A from Bacillus sp.
AH-42	Lipase B from Candida antarctica (liq)
AH-43	Lipase A from Candida antarctica
AH-44	Protease B from Bacillus sp.
AH-45	Lipase from Thermomyces lanuginosus
AH-46	Lipase C from Rhizomucor miehei

Typical Procedure - Aqueous hydrolysis of esters:

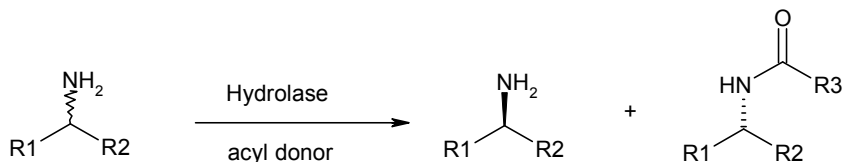


These bioresolutions are performed in neutral phosphate buffer. The exact composition of the aqueous system depends on the substrate physical form. If the ester is a liquid, a biphasic mixture of buffer and ester may suffice, but if it is a solid, it is more convenient to add a co-solvent to solubilise the ester. This may be a water miscible solvent creating a single phase system, or an immiscible solvent creating a biphasic system. 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 (920 mg) in a solvent such as MTBE (11.5 mL) 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.

Typical Procedure - Bioresolution of amines:

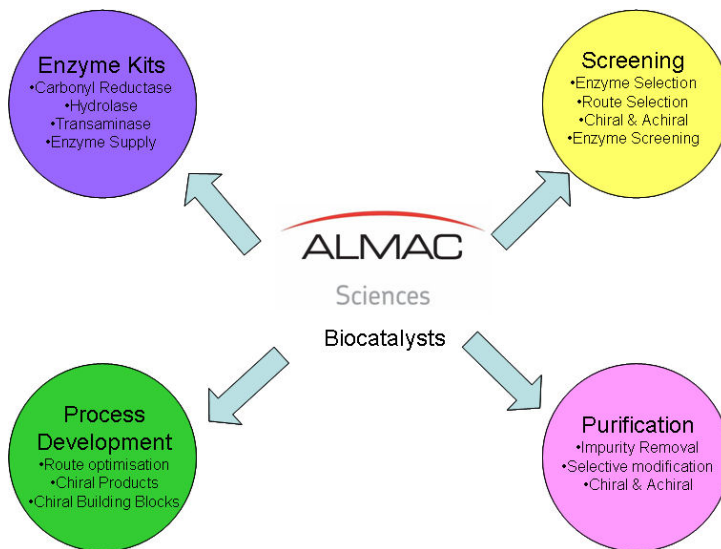


Amines are readily resolved by enzyme-catalysed acylation, providing a valuable alternative approach to crystallisation methods.

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 (1.38 g) in ethyl butyrate (69 mL) 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.
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.

Biocatalysis Services:



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