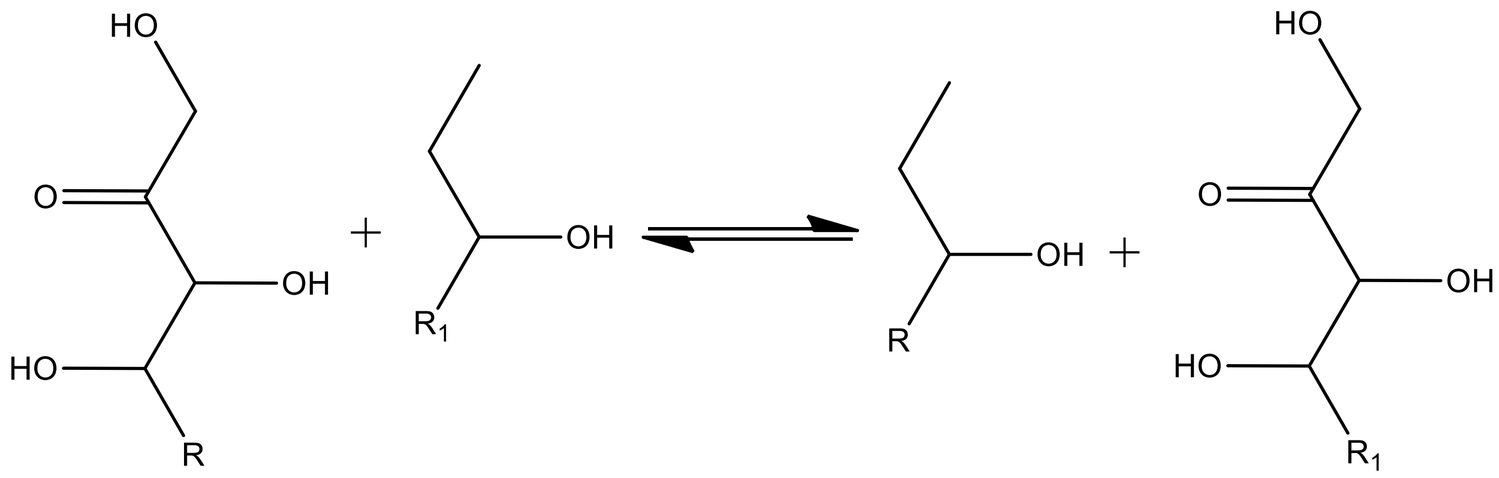
Transketolase (TKT) Enzyme Screening Kit

TKTESK-5000

Partnering to Advance Human Health

Applications

Transfer of a two-carbon ketol group from a ketose donor to an aldose acceptor, via a thiamine pyrophosphate intermediate.



Kit description

The kit contains 50 transketolase biocatalysts as lyophilised powders in bottle format, as well as pre-prepared Tris buffer and a ketose donor and aldose acceptor.

TKTs contained in the screening kit:

TKT-001	TKT-009	TKT-017	TKT-025	TKT-033	TKT-041	TKT-049
TKT-002	TKT-010	TKT-018	TKT-026	TKT-034	TKT-042	TKT-050
TKT-003	TKT-011	TKT-019	TKT-027	TKT-035	TKT-043	
TKT-004	TKT-012	TKT-020	TKT-028	TKT-036	TKT-044	
TKT-005	TKT-013	TKT-021	TKT-029	TKT-037	TKT-045	
TKT-006	TKT-014	TKT-022	TKT-030	TKT-038	TKT-046	
TKT-007	TKT-015	TKT-023	TKT-031	TKT-039	TKT-047	
TKT-008	TKT-016	TKT-024	TKT-032	TKT-040	TKT-048	

Contents

TKTs	50 enzymes (50 mg each)
Enzyme resuspension mix*	5 vials (65 mg)
Ketose donor (LiHPA)	5 vials (60mg)
Aldose acceptor (GO)	5 vials (60mg)
DMSO	1 vial (10 mL)
50 mM Tris buffer (pH 7.5)	1 bottle (60 mL)

*Once dissolved in 10 mL Tris buffer, enzyme resuspension mix contains 4 mg/mL $MgCl_2$ and 2.5 mg/mL thiamine pyrophosphate

LiHPA and GO are Lithium hydroxypyruvate and glycoaldehyde respectively

Screening Procedure- ketose donation

1. Label 50 x 1.5mL tubes with the enzyme names detailed in the kit contents and weigh 10g of the corresponding enzyme into each tube
2. Dissolve the enzyme suspension mix (1 vial) in 10 mL of Tris buffer.**
3. Once dissolved, add 100 µL of the enzyme resuspension solution to each vial containing 10 mg CRED.
4. Dissolve the ketose donor (1 vial) in 6mL Tris buffer
5. Once dissolved add 100 µL ketose donor to each tube
6. Add a solution of 5-10 mg substrate in DMSO (25-50 µL, depending on solubility).
7. Shake at room temperature (or ideally 30 °C). Agitate overnight.
8. Extract product with an organic solvent (MTBE, EtOAc etc.).
9. Analyse sample by GC/HPLC to determine conversion and product ee.

Screening Procedure- ketose removal

1. Label 50 x 1.5mL tubes with the enzyme names detailed in the kit contents and weigh 10g of the corresponding enzyme into each tube
2. Dissolve the enzyme suspension mix (1 vial) in 10 mL of Tris buffer.**
3. Once dissolved, add 100 µL of the enzyme resuspension solution to each vial containing 10 mg CRED.
4. Dissolve the ketose donor (1 vial) in 6mL Tris buffer
5. Once dissolved add 100 µL ketose acceptor to each tube
6. Add a solution of 5-10 mg substrate in DMSO (25-50 µL, depending on solubility).
7. Shake at room temperature (or ideally 30 °C). Agitate overnight.
8. Extract product with an organic solvent (MTBE, EtOAc etc.).
9. Analyse sample by GC/HPLC to determine conversion and product ee.

**It is recommended to make the reaction mix solution fresh and use immediately. Avoid storage of the reaction mix as a solution, as this will degrade over time.

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

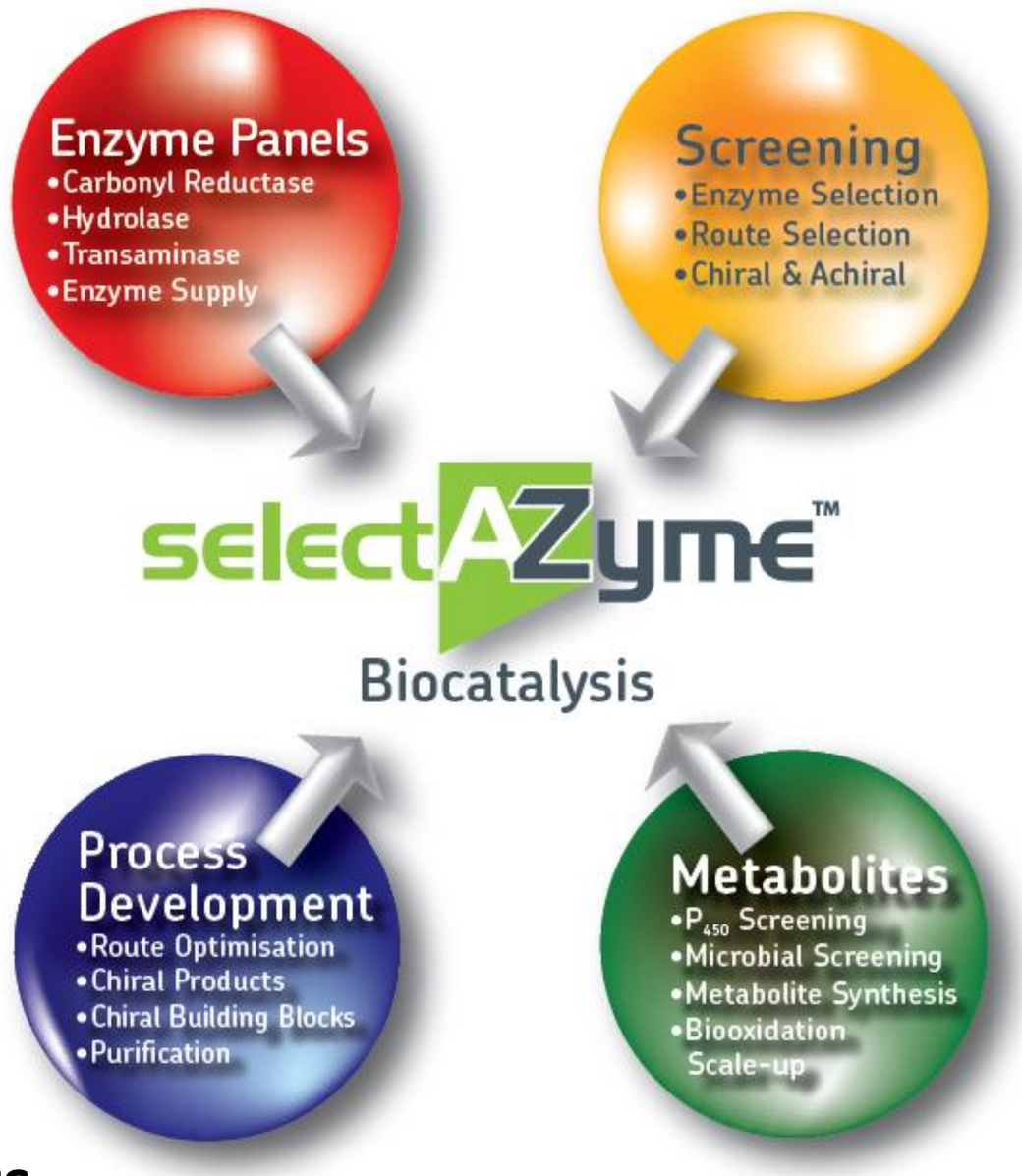
Partnering to Advance Human Health

Carbonyl Reductase (CRED) Enzyme Screening Kit

CESK-9600A

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 unique selectAZyme platform offers a range of enzymes suitable for carrying out a wide variety of chemical reactions. Our biocatalysts are prepared in easy to use kits for rapid customer evaluation without any IP issues. These include the following:

Carbonyl Reductase (CRED) biocatalysts

>300 CREDs for the production of chiral alcohols from pro-chiral ketones

Hydrolase biocatalysts

>100 hydrolases for selective hydrolysis in aqueous media, selective acylation in non-aqueous media, resolution of secondary alcohols, amines and thiols, formation of peptides

Nitrilase biocatalysts

>200 nitrilases for the synthesis of carboxylic acids by enzymatic hydrolysis of nitriles

Transaminase (TAm) biocatalysts

>200 TAms for the production of chiral amines by asymmetric synthesis from pro-chiral ketones or resolution of racemic amines

Ene Reductase (ERED) biocatalysts

>200 EREDs for asymmetric reduction of activated alkenes

For the full range of enzyme screening kits on offer, please check the Almac website

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.

Technical Contacts:

Prof. Tom Moody, Tel: +44 (0)28 3833 2200 Ext. 5517, E-mail: tom.moody@almacgroup.com.

Dr. Derek Quinn, Tel: +44 (0)28 3833 2200 Ext. 5833, E-mail: derek.quinn@almacgroup.com.

Address: Almac Biocatalysis & Isotope Chemistry Group,

20 Seagoe Industrial Estate, Craigavon BT63 5QD

Web: www.almacgroup.com

Email: biocatalysis@almacgroup.com