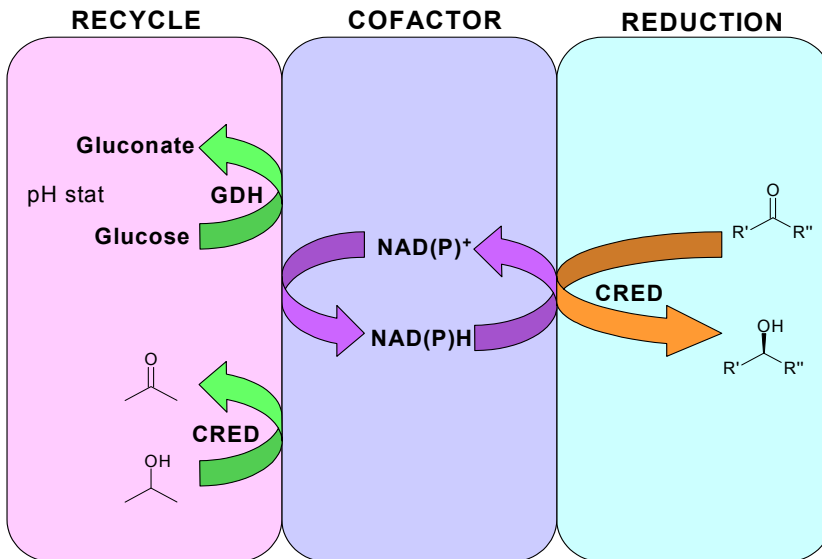


Carbonyl Reductase (CRED) Enzyme Screening Kit: CESK-4000

Applications:

Synthesis of enantiomerically pure (R) or (S) alcohols by enzymatic reduction of carbonyl compounds.



Kit Description:

The kit contains 40 diverse pre-formulated carbonyl reductase (CRED) biocatalysts as dry powders, as well as pre-prepared phosphate buffer ready for use, NAD and NADP cofactors, and glucose dehydrogenase (GDH) for the cofactor recycle system. Note that for some enzymes, it is possible to recycle cofactor using an alcohol donor such as isopropyl alcohol (IPA).

Contents:

CREDs	40 vials lyophilised powder (50 mg each)
NADP	1 vial (150 mg)
NAD	1 vial (150 mg)
GDH	1 vial (500 mg)
Glucose	1 vial (5 g)
DMSO	2 vials (2 x 10 mL)
0.1M KH ₂ PO ₄ buffer (pH 7.0)	1 bottle (200 mL)

An adequate supply of NADP, NAD, GDH, glucose, and buffer has been provided for 3 screens with each enzyme. Additional GDH, buffer, glucose or cofactors are available for purchase from Almac.

Storage:

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

Enzyme List and Cofactor preference:

In most cases CRED enzymes will accept both NADP and NAD as cofactors, but exhibit a preference for one over the other. These cofactor preferences are listed in the table below.

CRED	Cofactor
A101	NADP
A201	NADP
A301	NADP
A401	NADP
A501	NADP
A601	NADP
A701	NADP
A801	NADP
A901	NADP
A121	NADP
A131	NAD
A141	NADP
A151	NAD
A161	NAD
A171	NAD
A181	NADP
A191	NAD
A211	NADP
A221	NAD
A231	NADP

CRED	Cofactor
A241	NADP
A251	NAD
A261	NADP
A271	NADP
A281	NADP
A291	NADP
A311	NADP
A321	NADP
A331	NADP
A341	NADP
A351	NADP
A361	NADP
A371	NADP
A381	NADP
A391	NADP
N501	NADP
N701	NADP
N121	NADP
N131	NADP
N151	NADP

Typical Procedure – Reduction Reaction:

Reagents:

- A: 15 mg/mL solution of CRED in buffer.
- B: 300 mg/mL solution of glucose in buffer.
- C: 10 mg/mL solution of NADP in buffer.
- D: 10 mg/mL solution of NAD in buffer.
- E: 20 mg/mL solution of GDH in buffer.

Procedure:

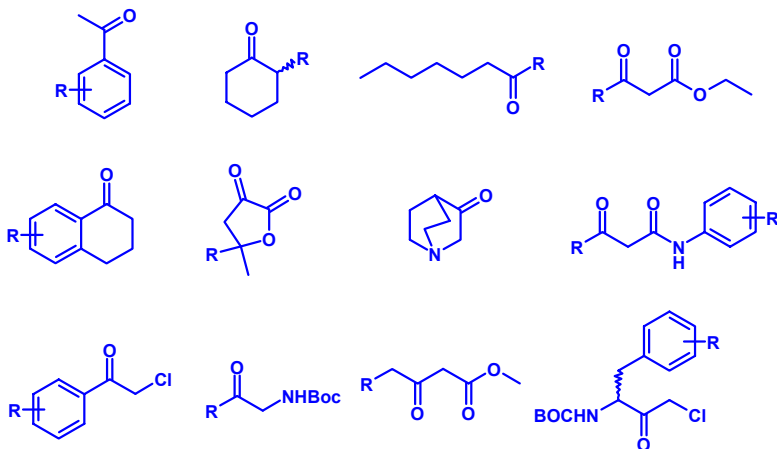
1. Into a flask/vial, add reagent A (1 mL).
2. Add reagent B (100 μ L).
3. Add reagent C or D (100 μ L), depending on enzyme preference (see cofactor preference table).
4. Add reagent E (100 μ L).
5. Add a solution of ~20 mg ketone substrate in organic solvent (50-150 μ L, depending on solubility) such as DMSO or MTBE.
6. Shake/stir at room temperature (or ideally 30 $^{\circ}$ C). Agitate overnight.
7. Extract product with an organic solvent (MTBE, EtOAc etc.).
8. Analyse sample by chiral GC/HPLC for conversion and product ee.

It is not advisable to keep stock solutions of cofactors or enzymes, as these will degrade over time. Make each stock solution fresh on the day of use.

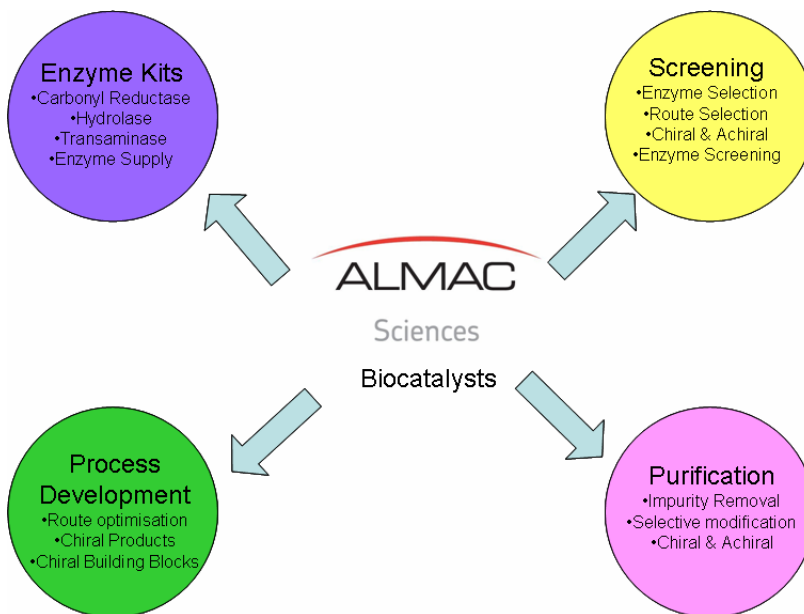
A sufficient supply of all contents has been provided for 3 screens with each enzyme. Additional components are available for purchase from Almac.

Substrate Range:

A wide variety of structurally diverse carbonyl compounds, including aliphatic & aromatic ketones, diketones, ketoesters, ketoamides, ketoacids, cyclic ketones and aldehydes. A selection is shown below.



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