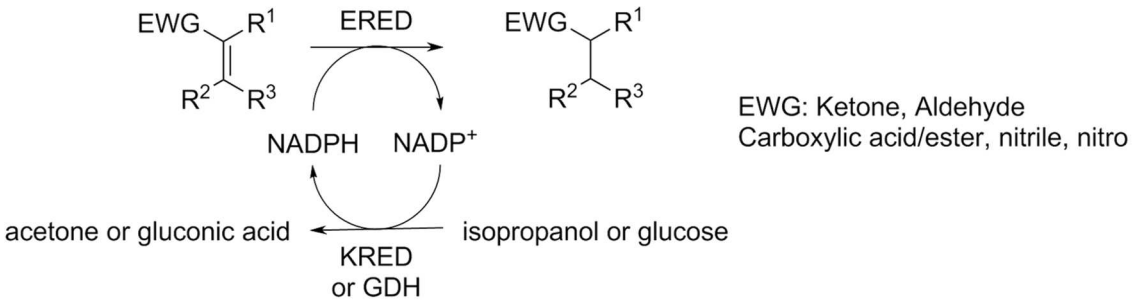
Codex® ERED Screening Kit

Screening Protocol

REACTION OF INTEREST



CODEX® ERED SCREENING KIT GENERAL INFORMATION

The Codex® ERED Screening Kit contains 7 ene reductases (EREDs) that can be screened to quickly determine the feasibility of using an ERED for an asymmetric CC double bond reduction.

To recycle the nicotinamide cofactor, a glucose dehydrogenase (GDH) with glucose can be utilized. The GDH enzyme converts glucose to gluconolactone which spontaneously hydrolyses to gluconic acid, making the reaction irreversible. This produces an equivalent of acid which may cause a pH drop depending on the conversion and buffer capacity.

Another alternative cofactor recycling system is a ketoreductase (KRED) with isopropanol, generating acetone as a product (not provided in this kit). When using the KRED system at scale, the reaction can be driven by removing the acetone product from the reaction. The choice of cofactor recycling system depends on whether the substrate is susceptible to the KRED (reduction of the carbonyl group).

Recommended storage temperature for the enzyme powders is -20 °C.

The 250 mg ERED Screening Kit contains sufficient enzyme to perform ~25 screens using the protocol given. Alternatively, fewer screens can be performed, and the remaining enzyme can be used for confirmation and optimization reactions.

CODEX® ERED SCREENING KIT CONTENTS

Item	Enzyme	Amount
1	ERED-103	250 mg
2	ERED-110	250 mg
3	ERED-112	250 mg
4	ERED-207	250 mg
5	ERED-P1-A04	250 mg
6	ERED-P1-E01	250 mg
7	ERED-P1-H09	250 mg
8	GDH-105 (GDH)	2.0 g
9	$NADP^+$	2.0 g

Additional components required, not supplied in the kit:

- 100 mM Potassium phosphate buffer, pH 7
- Glucose
- Alkene substrate
- Co-Solvent (such as DMSO or isopropanol), if necessary

CODEX® ERED SCREENING KIT SCREENING PROCEDURE WITH GDH COFACTOR RECYCLING SYSTEM

1. Weigh out approximately 10 mg of each ERED into separate, labelled vials (vials should have at least 1.5 mL total volume). Plastic conical centrifuge tubes of 2 mL volume work well for this as the reaction can be extracted or quenched in the same vial.
2. For each full screen, make a solution of 20 mg GDH-105, 2 molar equivalents of glucose (relative to substrate), 5 mg $NADP^+$ in 9 mL phosphate buffer. Mix thoroughly until all components are dissolved but do not sonicate the solution.
3. Dissolve ~50 mg of your alkene substrate in 1 mL DMSO or buffer.
4. Add the substrate solution to the GDH/cofactor buffer solution.
5. To start the reaction, add 1 mL of the resulting solution to each reaction vial containing an ERED.
6. Mix the reactions at 30 °C. This is best done by placing the vials horizontally in a shaker, but any method that gives good mixing is acceptable. Assay the reaction mixture after ~24 hours by any preferred method to monitor the conversion of alkene to alkane. A general work-up protocol is given below.

CODEX® ERED SCREENING KIT WORK-UP AND ANALYSIS

1. Depending on the anticipated method of analysis, for normal phase HPLC or GC add 1 mL ethyl acetate, isopropyl acetate, methyl *t*-butyl ether or other appropriate extraction solvent to each reaction. For reversed-phase HPLC analysis, quench the reaction by addition of 1 mL acetonitrile. Mix well to ensure that substrate and product have been fully solubilized or extracted.
2. Centrifuge each mixture at ~4000 rpm for 2 min to separate the phases and sediment any precipitated protein. If a centrifuge is not available, the quenched reaction can be filtered using a syringe filter or, for extractive workup, the phases can be allowed to separate unaided.
3. Transfer the organic phase or the aqueous acetonitrile phase from each quenched reaction vial to autosampler vials.
4. Analyze for conversion and selectivity by preferred method of analysis. Note: If analyzing by reversed-phase HPLC, there will often be a large peak early in the chromatogram corresponding to the NADP(H) cofactor.

WHAT TO DO IF…

- **… the substrate is very insoluble in water?**

  This is typically not an issue unless the substrate solubility is extremely low (essentially undetectable); a cloudy reaction mixture is acceptable. It is important the substrate is added equally to each enzyme. If this cannot be done from an aqueous or IPA stock solution, it can be added neat to each reaction vial.

- **… low or no activity is found?**

  Allow the reaction to run for a longer time, increase the temperature (40 °C) and/or increase the enzyme concentration.

- **… there are too many hits and differences among them cannot be easily determined?**

  Stop the reaction at an earlier time or repeat the screen using a higher substrate loading. For the latter, maintain DMSO concentration at <10% v/v.

- **… I want to practice performing a positive control reaction before running my test substrates?**

  Cyclohexenone is known as a good substrate for ERED-P1-E01 and you can follow the protocol above for a practice run.

- **… I have other questions?**

  Feel free to contact us at sales@codexis.com and we will be happy to assist you

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