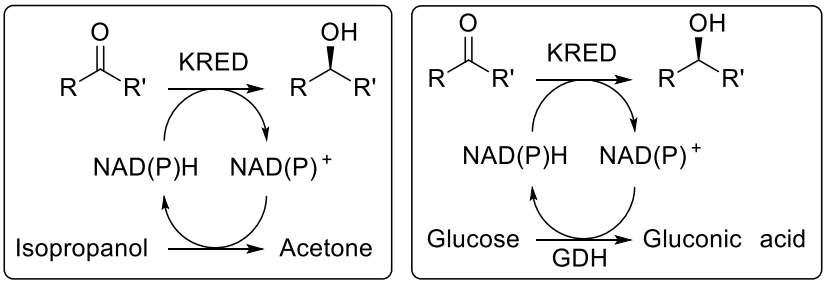
Codex® KRED Screening Kit

Screening Protocol

THE KRED REACTION



CODEX® KRED SCREENING KIT GENERAL INFORMATION

- The Codex® KRED Screening Kit contains 24 ketoreductase (KRED) enzymes that have been curated for their broad substrate range, high and diverse stereoselectivity, and high stability from more than 70 projects. This kit is a useful tool to quickly determine the feasibility of using a KRED for an asymmetric ketone reduction.

- Nicotinamide cofactor, NADPH or NADH, is used as the direct hydride source. To avoid using stoichiometric amounts of cofactor and to drive the equilibrium, the cofactor is recycled *in situ*. Most of the enzymes in the Codex® KRED Screening Kit accept NADPH as the cofactor and can utilize isopropanol as a reductant to convert the oxidized cofactor back to the reduced form. The presence of isopropanol is also useful in dissolving substrates with limited aqueous solubility. These enzymes are listed in the following table under Screening Procedure P.

- There are some enzymes in the Codex® KRED Screening Kit that do not readily accept isopropanol, and these are listed in the following table under Screening Procedure N. For these enzymes, glucose is used as the reductant to convert the oxidized cofactor back to the reduced form. This reaction is catalyzed by a second enzyme, glucose dehydrogenase (GDH), which is included in the KRED Recycle Mix N.

- The recommended storage temperature for the enzyme powders −20 °C.

- The Codex® KRED Screening Kit contains sufficient enzyme (250 mg each) and recycle mix to perform at least 20 screens using the protocol given. Alternatively, fewer screens can be performed and the remaining enzyme and recycle mix can be used for confirmation and optimization reactions.

Codexis, Inc.
200 Penobscot Drive, Redwood City, CA 94063 USA
www.codexis.com | sales@codexis.com | Phone: +1 (650) 421-8100

CODEX® KRED SCREENING KIT CONTENTS

Item	Enzyme	Amount	Screening Procedure	Cofactor	Cofactor Recycling System
1	KRED-P1-B02	250 mg	P	NADPH	Isopropanol
2	KRED-P1-B05	250 mg	P	NADPH	Isopropanol
3	KRED-P1-B10	250 mg	P	NADPH	Isopropanol
4	KRED-P1-B12	250 mg	P	NADPH	Isopropanol
5	KRED-P2-B02	250 mg	P	NADPH	Isopropanol
6	KRED-P2-C02	250 mg	P	NADPH	Isopropanol
7	KRED-P2-D11	250 mg	P	NADPH	Isopropanol
8	KRED-P2-G03	250 mg	P	NADPH	Isopropanol
9	KRED-P2-H07	250 mg	P	NADPH	Isopropanol
10	KRED-P3-B03	250 mg	P	NADPH	Isopropanol
11	KRED-P3-G09	250 mg	P	NADPH	Isopropanol
12	KRED-244	250 mg	P	NADPH	Isopropanol
13	KRED-264	250 mg	P	NADPH	Isopropanol
14	KRED-459	250 mg	P	NADPH	Isopropanol
15	KRED-462	250 mg	P	NADPH	Isopropanol
16	KRED-463	250 mg	P	NADPH	Isopropanol
17	KRED-464	250 mg	P	NADPH	Isopropanol
18	KRED-465	250 mg	P	NADPH	Isopropanol
19	KRED-460	250 mg	P	NAD(P)H	Isopropanol
20	KRED-461	250 mg	N	NAD(P)H	GDH/glucose
21	KRED-101	250 mg	N	NADPH	GDH/glucose
22	KRED-119	250 mg	N	NADPH	GDH/glucose
23	KRED-130	250 mg	N	NADPH	GDH/glucose
24	KRED-NADH-110	250 mg	N	NADH	GDH/glucose
25	KRED Recycling mix N[1]	10 g	N	N/A	GDH/glucose
26	KRED Recycling mix P[2]	20 g	P	N/A	Isopropanol

[1]The reconstituted KRED Recycle Mix N contains 263 mM sodium phosphate, 1.7 mM magnesium sulfate, 1.1 mM $NADP^+$, 1.1 mM NAD^+, 80 mM D-glucose, 4.3 U/mL glucose dehydrogenase, pH 7.0.

[2]The reconstituted KRED Recycle Mix P contains 128 mM sodium phosphate, 1.7 mM magnesium sulfate, 1.1 mM $NADP^+$, pH 7.0.

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CODEX® KRED SCREENING KIT—SUGGESTED SCREENING PROCEDURE

Steps	Procedure P (KREDs 1–19)	Procedure N (KREDs 20–24)
① KRED Dispensing	• Weigh out ~10 mg of each KRED in the Codex® KRED Screening Kit into separate labelled vials – 2 mL centrifuge tubes work well as the reaction can be extracted or quenched in the same tube. Alternatively, small glass vials with magnetic stirring can also be used.	
② Recycle Mix Reconstitution*	• Add 15 mL deionized water to 300 mg dry **KRED Recycle Mix P** for each full screen. Mix well until all solids are dissolved. • Add 1.5 mL isopropanol to above solution and mix well.	• Add 3 mL deionized water to 150 mg dry **KRED Recycle Mix N** for each full screen. Mix well until all solids are dissolved (do not add isopropanol to this solution).
③ Substrate Solution	• Dissolve ~100 mg of your ketone substrate into 0.9 mL of DMSO. • If ketone is not soluble in DMSO, water, buffer or 50% DMSO in water can be used instead.	
④ Reaction Initiation	• Add 475 µL of the reconstituted **KRED Recycle Mix P containing isopropanol** to each vial containing a KRED. • Add 25 µL of the substrate solution in DMSO to each vial.	• Add 475 µL of the reconstituted **KRED Recycle Mix N** to each vial containing a KRED. • Add 25 µL of the substrate solution in DMSO to each vial.
⑤ Overnight Incubation	• Incubate the reactions for ~24 hours at 30 °C while shaking. Shaking can be achieved by using a tube shaker, magnetic stirring, by placing the vials horizontally in an orbital shaker, or by any other method that provides good mixing.	
⑥ Reaction Workup and Analysis	• **Suggested workup for analysis by reversed phase HPLC:** Quench the reactions by adding 1 mL of acetonitrile, mix well for several minutes and centrifuge to sediment denatured proteins and other insolubles. If necessary, dilute the quenched clarified samples in 50% acetonitrile in water prior to analysis. • **Suggested workup for analysis by normal phase HPLC or GC:** Quench and extract the reactions by adding 1 mL of an organic solvent such as methyl *tert*-butyl ether, ethyl acetate, or similar. Mix well and centrifuge to separate the phases. Remove the organic layer for analysis.	

*Recycle Mix Reconstitution: Prepare fresh solution each time to avoid decomposition of the cofactor.

CODEX® KRED SCREENING KIT – FINAL CONCENTRATION OF REACTION COMPONENTS

Component	Procedure P	Procedure N
KRED (g/L)	20	20
Ketone substrate (g/L)	5	5
DMSO (%)	4.5	4.5
Isopropanol (%)	9	Not present
$MgSO_4$ (mM)	1.6	1.6
$NADP^+$ (mM)	1.0	1.0
NAD^+ (mM)	Not present	1.0
D-glucose (mM)	Not present	76
GDH (Units/mL)	Not present	4.1
Sodium phosphate (mM)	122	250

Please see the Codex® Screening Kit FAQs for any additional questions or contact us at sales@codexis.com

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