

## Ene-reductases SEQENZYM kit

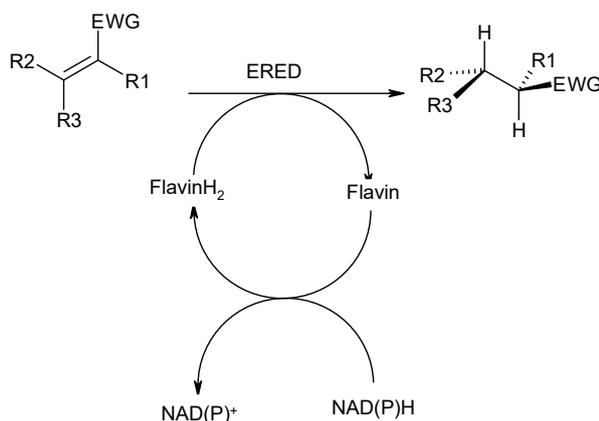
### Technical Data Sheet

#### GENERAL INFORMATION

The SEQENZYM™ - Ene-Reductase Kit contains 8 enzymes that have been selected for their selectivity and their wide range of potential substrates.

Ene-reductases (ERED) are flavin-dependent enzymes catalyzing the stereoselective reduction of activated C=C bonds at the expense of a nicotinamide cofactor. They have been applied to the synthesis of valuable enantiopure products, including chiral building blocks with broad industrial applications, terpenoids, amino acid derivatives and fragrances.

#### Reduction of a C=C bond with an Ene-reductase



EWG = activating electron-withdrawing group :  
aldehyde, ketone, imine, nitro, carboxylic acid or ester  
chiral center

Efficient cofactors recycling procedures have been developed by Protéus, please contact us for additional information.

#### KIT DESCRIPTION

The kit contains 8 ene-reductases as cell-free extracts for R&D use only (E4390, E4453, E4454, E4455, E4456, E4457, E4458, E4459, 50 mg each). Cofactor NADPH (β-Nicotinamide-Adenine Dinucleotide Phosphate, Reduced, CAS 2646-71-16, 140 mg) and NADH (β-Nicotinamide adenine dinucleotide, reduced disodium salt, CAS 606-68-8, 45 mg) are provided. The screening kit contains sufficient enzyme and cofactors to perform 5

assays per enzyme at 1 mL scale. Buffer salts are not provided; for buffer preparation, see “ADDITIONAL INFORMATION”.

Enzyme	Optimal temperature	Cofactor	Buffer
E4390	50 °C	NADPH	100 mM sodium phosphate pH 7
E4453	30 °C	NADPH	100 mM sodium phosphate pH 7
E4454	30 °C	NADPH	100 mM sodium phosphate pH 7
E4455	30 °C	NADPH	100 mM sodium phosphate pH 7
E4456	30 °C	NADPH	100 mM sodium phosphate pH 7
E4457	30 °C	NADH	100 mM sodium phosphate pH 7
E4458	50 °C	NADH	100 mM sodium phosphate pH 7
E4459	50 °C	NADPH	100 mM sodium phosphate pH 7

### SCREENING PROCEDURE FOR ENE-REDUCTASES

The following conditions are test conditions that can be optimized during further development steps – **Contact us for more details.**

1. Stock solutions preparation for a full screen (assessment of the 8 ERED):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
NADH	8 mg	1.1 mL of 100 mM sodium phosphate pH 7	500 µL	
NADPH	27 mg	3.2 mL of 100 mM sodium phosphate pH 7	500 µL	
Substrate	0.045 mmol	0.9 mL of 100 mM sodium phosphate pH 7	100 µL	
ERED	10 mg	400 µL of 100 mM sodium phosphate pH 7	400 µL	Homogenize well with pipet*

\* : Take care to well suspend the cell-free extracts to get an homogeneous solution. Do not sonicate.

2. In a vial, mix 500 µL of NADH or NADPH with 100 µL of substrate.
3. Start the reaction with the addition of 400 µL of the solution of ERED. Heat the reaction mixture at the required temperature (see table - kit description) under magnetic agitation.
4. After approximately 24 hours, analyze the reaction by any preferred method to determine the conversion of the alkene to the target reduced product.

## STORAGE

Recommended storage temperature for the enzymes and cofactors is -20 °C.

Prepare freshly cofactor and enzyme suspensions before use.

## ADDITIONAL INFORMATION

Preparation of 100 mM sodium phosphate pH 7 buffer:

Mix 819 mg of Na<sub>2</sub>HPO<sub>4</sub> and 507 mg of NaH<sub>2</sub>PO<sub>4</sub>, dissolve in H<sub>2</sub>O and complete to 100 mL.

## AFTER YOUR FIRST TRIALS,

**Protéus by Seqens** is available for any discussion concerning your results and further steps. Contact us if you need:

- Enlarge your screening with additional enzymes from our exclusive collection
- Larger enzyme quantities for your trials and scale-up
- Performance optimization on your chemistry: parameters of enzyme use

Protéus-by-Seqens has powerful tools to improve the enzyme performance and activity: our expertise is the fine-tuning of biocatalysts by directed evolution using Protéus-by-Seqens proprietary methodology (Evosight™ or L-Shuffling™) – **Contact us for more details.**

Keep in mind that Protéus by Seqens is dedicated to the development of biocatalyzed reactions and offers industrial scale-up capabilities within CDMO facilities – **Contact us for a quote.**

## CONTACT

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