Alcohol Dehydrogenases SEQENZYM kit

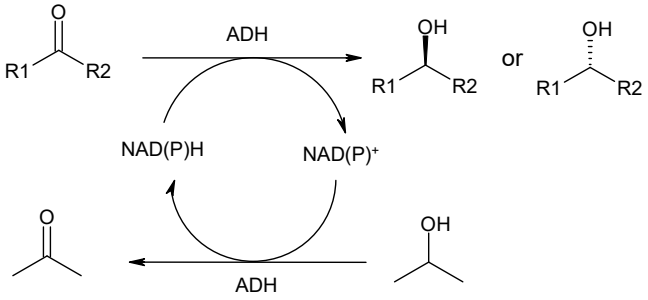
Technical Data Sheet

GENERAL INFORMATION

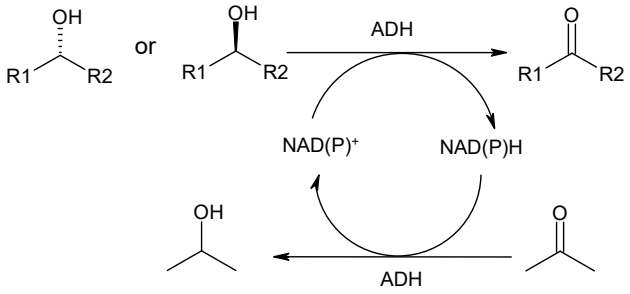
The SEQENZYM™ - Alcohol Dehydrogenases Kit contains 10 alcohol dehydrogenases (ADH) that have been selected for their selectivity and for their wide range of potential substrates.

Alcohol Dehydrogenases are used for the oxidation of alcohols to ketones, including kinetic resolution of racemic alcohols, or for the reduction of ketones to alcohols. Because these enzymes require cofactors (NAD(P)H) that are too expensive to be used stoichiometrically, the latter must be regenerated. Cofactor enzymatic regeneration is carried out in tandem with the transformation of substrate, in a one-pot reaction. Thus, to regenerate the cofactors, a sacrificial cosubstrate is converted by the same ADH in the opposite redox direction. An excess of *iso*propanol (IPA) is needed to reduce the ketone into the alcohol while the oxidation reaction requires an excess of acetone to convert the alcohol to the corresponding ketone.

Reduction of a ketone with an Alcohol Dehydrogenase



Oxidation of an alcohol with an Alcohol Dehydrogenase



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

KIT DESCRIPTION

The kit contains 10 alcohol dehydrogenases as cell-free extracts for R&D use only (E3373, E3378, E3627, E3653, E3666, E4336, E4337, E4391, E4408, E4410, 50 mg each). Cofactor $NADP^+$ (β-Nicotinamide-Adenine Dinucleotide Phosphate, Monosodium salt, Oxidized, CAS 1184-16-3, 138 mg) and NAD^+ (β-Nicotinamide-Adenine Dinucleotide, Oxidized, CAS 53-84-9, 52 mg) are provided. The screening kit contains sufficient enzyme and cofactors to perform 5 assays per enzyme at 1 mL scale. Buffer salts, IPA and acetone are not provided; for buffer preparation, see "ADDITIONAL INFORMATION".

Enzyme	Temperature	Required cofactor	Buffer
E3373	30 °C	$NADP^+$	100 mM sodium phosphate pH 7
E3378	50 °C	$NADP^+$	100 mM sodium phosphate pH 7
E3627	50 °C	$NADP^+$	100 mM sodium phosphate pH 7
E3653	30 °C	$NADP^+$	100 mM sodium phosphate pH 7
E3666	50 °C	NAD^+	100 mM sodium phosphate pH 7
E4336	30 °C	$NADP^+$	100 mM sodium phosphate pH 7
E4337	30 °C	$NADP^+$	100 mM sodium phosphate pH 7
E4391	30 °C	$NADP^+$	100 mM sodium phosphate pH 7
E4408	30 °C	NAD^+	100 mM sodium phosphate pH 7
E4410	30 °C	NAD^+	100 mM sodium phosphate pH 7

SCREENING PROCEDURE FOR REDUCTION

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 10 ADH):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
$NADP^+$	27.5 mg	360 µL of 100 mM sodium phosphate pH 7	50 µL	
NAD^+	10.3 mg	160 µL of 100 mM sodium phosphate pH 7	50 µL	
Substrate ketone	0.11 mmol	550 µL of IPA	50µL	
ADH	10 mg	900 µL of 100 mM sodium phosphate pH 7	900 µL	Homogeneize 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 50 µL of $NADP^+$ or NAD^+ solution (see table - kit description) with 50 µL of substrate.
3. Start the reaction with the addition of 900 µL of the solution of ADH. 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 ketone to the target alcohol and the enantiomeric excess.

SCREENING PROCEDURE FOR OXIDATION

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 10 ADH):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
$NADP^+$	27.5 mg	360 µL of 100 mM sodium phosphate pH 7	50 µL	
NAD^+	10.3 mg	160 µL of 100 mM sodium phosphate pH 7	50 µL	
Substrate alcohol	0.11 mmol	550 µL of acetone	50µL	
ADH	10 mg	900 µL of 100 mM sodium phosphate pH 7	900 µL	Homogeneize 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 50 µL of $NADP^+$ or NAD^+ solution (see table - kit description) with 50 µL of substrate.
3. Start the reaction with the addition of 900 µL of the solution of ADH. 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 alcohol to the target ketone and the enantiomeric excess.

STORAGE

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

Prepare freshly cofactors and enzyme suspensions before use.

ADDITIONAL INFORMATION

Preparation of 100 mM Sodium phosphate pH 7 buffer:

Mix 819 mg of Na_2HPO_4 and 507 mg of NaH_2PO_4, dissolve in H_2O 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.**

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CONTACT

+33(0)466 70 64 64

http://www.proteus.seqens.com

seqenzym@seqens.com

Dehalogenases SEQENZYM kit

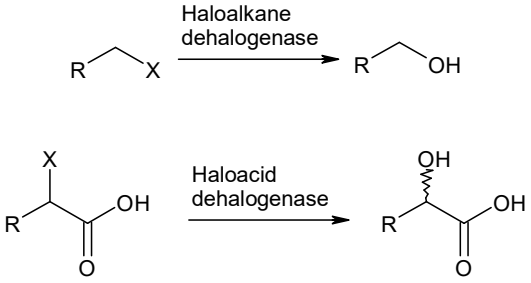
Technical Data Sheet

GENERAL INFORMATION

The SEQENZYM™ - Dehalogenase Kit contains 4 enzymes that have been selected for their selectivity and their wide range of potential substrates.

The 2-haloacid dehalogenases catalyse the hydrolytic dehalogenation of 2-haloalkanoic acids to produce 2-hydroxyalkanoic acids. They are only active on compounds in which the halogen is attached at the C2 position.

Haloalkane dehalogenases catalyse the hydrolysis of carbon-halogen bonds of halogenated compounds, to produce an alcohol. These enzymes are active toward halogenated alkanes, cycloalkanes, alkenes, ethers, alcohol, ketones or cyclic dienes. Hydrolytic cleavage of a carbon-halogen bond proceeds by the SN2 mechanism with the addition of water. Water is the only co-factor required for catalysis.



The D-haloacid dehalogenase E3397 catalyses the reaction of dehalogenation of the (R)-α-haloacid and can be used for the resolution of DL-α-haloacid to obtain the (S)-α-haloacid and the (R)-α-hydroxyacid or inversely in terms of stereoselectivity.

The L-haloacid dehalogenase E3528 catalyses the reaction of dehalogenation of the (S)-α-haloacid and it can be used for resolution of DL-α-Haloacid to obtain the (R)-α-haloacid and/or the (R)-α-hydroxyacid.

The haloalkane dehalogenase E4393 is able to dehalogenate stereoselectively various substrates to obtain halogenated or hydroxylated chirales derivatives. This enzyme accept various solvents (Alkanes, ethers) and is effective in biphasic medium.

The DL-haloacid dehalogenase E4426 is not steroselective and can be used to dehalogenate a DL-α-haloacid with a chain length of the alkyl group up to C8.

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KIT DESCRIPTION

The kit contains 4 dehalogenases as cell-free extracts for R&D use only (E3397, E3528, E4393, E4426, 50mg each). The screening kit contains sufficient enzyme and cofactors to perform 5 assays per enzyme at 1 mL scale. Buffer salts, H_2SO_4 and MTBE are not provided; for buffer preparation, see "ADDITIONAL INFORMATION".

Enzyme	Enzyme type	Selectivity	Temperature	Buffer
E3397	Haloacide dehalogenase	S	30 °C	200 mM TRIS-SO_4 pH 10.5
E3528	Haloacid dehalogenase	R	50 °C	200 mM TRIS-SO_4 pH 9.5
E4393	Haloalkane dehalogenase	-	40 °C	200 mM TRIS-SO_4 pH 9.5
E4426	Haloacid dehalogenase	Rac	20 °C	200 mM TRIS-SO_4 pH 8.5

SCREENING PROCEDURE FOR DEHALOGENASES

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 4 dehalogenases):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
Halogenated substrate	0.044 mmol	2.2 mL of MTBE	500 µL	
Dehalogenases	10 mg	500 µL of 0.2 M TRIS-SO_4 pH of the enzyme	500 µL	Homogeneize well with pipet*

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

2. In a vial, mix 500 µL of dehalogenase suspension with 500 µL of substrate. The reaction mixture appears as a biphasic system.

3. Heat the reaction mixture at the corresponding 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 halogenated substrate to the target alcohol and the enantiomeric excess.

STORAGE

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

Prepare freshly cofactor and enzyme suspensions before use.

ADDITIONAL INFORMATION

Preparation of 200 mM TRIS (tris(hydroxymethyl) aminomethane) buffer:

Dissolve 2.42 g of Tris(hydroxymethyl)aminomethane in 80 mL of H_2O. Adjust the pH with a solution of H_2SO_4 1 N and complete to 100 mL with H_2O.

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

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CONTACT

+33(0)466 70 64 64

http://www.proteus.seqens.com

seqenzym@seqens.com

Ene-reductases SEQENZYM kit

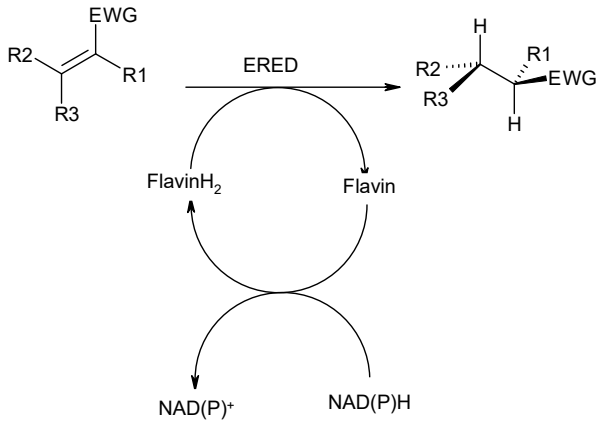
Technical Data Sheet

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	Homogeneize 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_2HPO_4 and 507 mg of NaH_2PO_4, dissolve in H_2O and complete to 100 mL.

AFTER YOUR FIRST TRIALS,

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CONTACT

+33(0)466 70 64 64

http://www.proteus.seqens.com

seqenzym@seqens.com

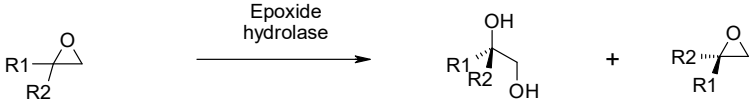
Epoxide Hydrolases SEQENZYM kit

Technical Data Sheet

GENERAL INFORMATION

The SEQENZYM™ - Epoxide Hydrolases Kit contains 4 epoxide hydrolases that have been selected for their selectivity and for their wide range of potential substrates.

Epoxide hydrolases catalyse the opening of oxirane rings by water, generating vicinals diols as products. When the starting epoxide is racemic, the epoxide hydrolase can selectively recognize one of the two enantiomers of the substrate allowing the kinetic resolution of the racemic mixture. Note that different substitution patterns could be tolerated and therefore different regioselectivity could be observed.



KIT DESCRIPTION

The kit contains 4 epoxide hydrolases as cell-free extracts for R&D use only (E3897, E3898, E4420 and E4421, 50 mg each). The screening kit contains sufficient enzyme amount to perform 5 assays per enzyme at 1 mL scale. Buffer salts and DMSO are not provided; for buffer preparation, see “ADDITIONAL INFORMATION”.

SCREENING PROCEDURE FOR EPOXIDE HYDROLASES

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 4 epoxide hydrolases):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
Substrate	0.10 mmol	250 µL of DMSO	50 µL	
Epoxide hydrolase	10 mg	950 µL of 100 mM sodium phosphate pH 7	950 µL	Homogeneize well with pipet*

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

2. In a vial, mix 950 µL of the suspension of epoxide hydrolase with 50 µL of the solution of substrate.

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3. Heat the reaction mixture at 30 °C under magnetic agitation.

4. Monitor the reaction over time by any preferred method to determine the conversion and the enantiomeric excess.

STORAGE

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

Prepare freshly enzyme suspensions before use.

ADDITIONAL INFORMATION

Preparation of 100 mM sodium phosphate pH 7 buffer:

Mix 819 mg of Na_2HPO_4 and 508 mg of NaH_2PO_4, dissolve in H_2O and complete to 100 mL.

AFTER YOUR FIRST TRIALS,

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http://www.proteus.seqens.com

seqenzym@seqens.com

Lipases and Esterases SEQENZYM kit

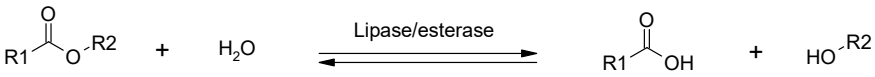
Technical Data Sheet

GENERAL INFORMATION

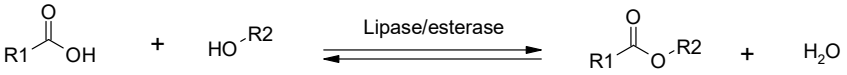
The SEQENZYM™ - Lipases and Esterases Kit contains 9 enzymes that have been selected for their broad substrate range and for their high selectivity and stability.

Esterases and lipases catalyze the hydrolysis of esters, the esterification of acids and the transesterification of esters. They do not require any cofactor, are generally stable and tolerate high ratios of organic solvents.

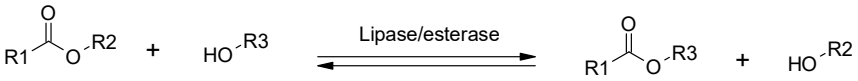
Hydrolysis reaction



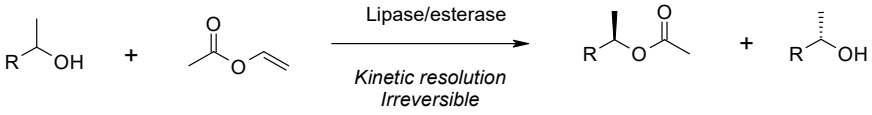
Esterification reaction



Transesterification reaction



Due to their enantioselectivity, these enzymes allow for efficient kinetic resolutions of racemic mixtures. Furthermore, the esterase-catalyzed transesterification reaction of vinyl esters with alcohols works well since the reaction forms acetaldehyde which is not a substrate for the enzyme. Therefore, the equilibrium of the reaction could easily be switched towards the desired reaction.



KIT DESCRIPTION

The kit contains 9 esterases as cell-free extracts for R&D use only (E3576, E3769, E4197, E4394, E4395, E4396, E4397, E4398, E4399, 50 mg each). The screening kit contains sufficient enzyme amount to perform 5 assays per enzyme at 1 mL scale. Buffer salts, alcohols and solvents are not provided; for buffer preparation, see “ADDITIONAL INFORMATION”.

70, allée Graham Bell - Parc Georges Besse - 30035 Nîmes cedex 1 - France - Tél.: +33(0)466 70 64 64 - Fax +33(0)466 70 64 60 - seqensrdnimesinfo@seqens.com - www.proteus.seqens.com

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Enzyme	Temperature	Buffer, pH
E3576	30 °C	100 mM sodium phosphate pH 8
E3769	30 °C	100 mM sodium phosphate pH 6
E4197	70 °C	100 mM sodium phosphate pH 7
E4394	80 °C	100 mM sodium phosphate pH 8
E4395	50 °C	100 mM sodium phosphate pH 7
E4396	70 °C	100 mM sodium phosphate pH 7
E4397	50 °C	100 mM sodium phosphate pH 7
E4398	70 °C	100 mM sodium phosphate pH 6
E4399	80 °C	100 mM sodium phosphate pH 7

SCREENING PROCEDURE FOR HYDROLYSIS OF A TARGET ESTER

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 9 lipases/esterases):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
Substrate	0.20 mmol	500 µL of DMSO	50 µL	
Lipase/esterase	10 mg	950 µL of buffer (see table)	950 µL	Homogeneize well with pipet*

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

2. In a vial, mix 950 µL of the suspension of lipase/esterase with 50 µL of the solution of substrate.

3. Heat the reaction mixture at the required temperature (see table - kit description) under magnetic agitation.

4. Monitor the reaction over time by any preferred method to determine the conversion and the enantiomeric excess.

SCREENING PROCEDURE FOR ESTERIFICATION OF A TARGET ACID

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 9 lipases/esterases):

	Stock solutions		Volume to add for each assay
	Amount	Dissolve in	
Acid substrate	0.20 mmol	5 mL of solvent*	500 µL
Alcohol	0.40 mmol	5 mL of solvent*	500 µL

*The solvent could be MTBE, toluene, methyl isopropyl ketone or an alkane.

2. In a vial, mix 10 mg of lipase/esterase in 500 µL of solution of substrate and 500 µL of solution of alcohol. Take care to well suspend the powder to get an homogeneous suspension. Do not sonicate.

3. Heat the reaction mixture at the required temperature (see table - kit description) under magnetic agitation.

4. Monitor the reaction over time by any preferred method to determine the conversion and the enantiomeric excess.

SCREENING PROCEDURE FOR TRANSESTERIFICATION OF A VINYL ESTER BY A TARGET ALCOHOL

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

1. Stock solution preparation for a full screen (assessment of the 9 lipases/esterases):

	Stock solutions		Volume to add for each assay
	Amount	Dissolve in	
Alcohol substrate	0.20 mmol	10 mL of vinyl ester*	1 mL

* Alternatively, the alcohol substrate can also be diluted in 10% V/V solution of vinyl ester in a solvent. The solvent could be MTBE, toluene, methyl isopropyl ketone or an alkane.

2. In a vial, mix 10 mg of lipase/esterase in 1 mL of solution of substrate. Take care to well suspend the powders to get an homogeneous suspension. Do not sonicate.

3. Heat the reaction mixture at the required temperature (see table - kit description) under magnetic agitation.

4. Monitor the reaction over time by any preferred method to determine the conversion and the enantiomeric excess.

STORAGE

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

Prepare freshly enzyme suspensions before use.

ADDITIONAL INFORMATION

Preparation of 100 mM sodium phosphate pH 6 buffer:

Mix 170 mg of Na_2HPO_4 and 1.214 mg of NaH_2PO_4, dissolve in H_2O and complete to 100 mL.

Preparation of 100 mM sodium phosphate pH 7 buffer:

Mix 819 mg of Na_2HPO_4 and 508 mg of NaH_2PO_4, dissolve in H_2O and complete to 100 mL.

Preparation of 100 mM sodium phosphate pH 8 buffer:

Mix 1.323 g of Na_2HPO_4 and 82 mg of NaH_2PO_4, dissolve in H_2O 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

+33(0)466 70 64 64

http://www.proteus.seqens.com

seqenzym@seqens.com

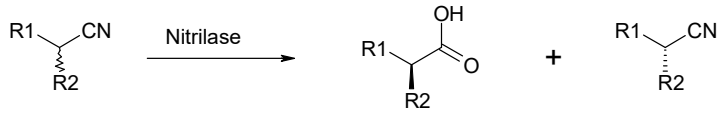
Nitrilases SEQENZYM kit

Technical Data Sheet

GENERAL INFORMATION

The SEQENZYM™ - Nitrilases Kit contains 9 nitrilases that have been selected for their selectivity and for their wide range of potential substrates.

Nitrilases catalyse the hydrolysis of nitriles to carboxylic acids and ammonia. They are used for the resolution of racemic mixtures. When the starting nitrile is racemic, the enzymes can selectively recognize one of the two enantiomers of the substrate and lead to a maximum yield of 50% in a kinetic resolution process.



R1 and R2 = Alkyl, Aryl or H

KIT DESCRIPTION

The kit contains 9 nitrilases as cell-free extracts for R&D use only (E4402, E4403, E4404, E4405, E4407, E4439, E4440, E4441 and E4442, 50 mg each). The screening kit contains sufficient enzyme amount to perform 5 assays per enzyme at 1 mL scale. Buffer salts and DMSO are not provided; for buffer preparation, see "ADDITIONAL INFORMATION".

SCREENING PROCEDURE FOR NITRILASES

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 9 nitrilases):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
Substrate	0.20 mmol	500 µL of DMSO	50 µL	-
Nitrilase	10 mg	950 µL of 100 mM sodium phosphate pH 7	950 µL	Homogeneize well with pipet*

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

70, allée Graham Bell - Parc Georges Besse - 30035 Nîmes cedex 1 - France - Tél.: +33(0)466 70 64 64 - Fax +33(0)466 70 64 60 - seqensrdnimesinfo@seqens.com - www.proteus.seqens.com

Protéus, SA - Société anonyme au capital de €187.471,35 - RCS Evry B 418 623 138 - SIRET 418 623 138 00041 - APE 7211Z - N° TVA : FR47418623138

Siège social/headquarter: 21, chemin de la sauvegarde – 21 Ecully Parc – CS 33167 - 69134 ECULLY Cedex - FRANCE

2. In a vial, mix 950 µL of the suspension of nitrilase with 50 µL of the solution of substrate.

3. Heat the reaction mixture at 30 °C under magnetic agitation.

4. Monitor the reaction over time by any preferred method to determine the conversion and the enantiomeric excess.

STORAGE

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

Prepare freshly enzyme suspensions before use.

ADDITIONAL INFORMATION

Preparation of 100 mM sodium phosphate pH 7 buffer:

Mix 819 mg of Na_2HPO_4 and 508 mg of NaH_2PO_4, dissolve in H_2O 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.**

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http://www.proteus.seqens.com

seqenzym@seqens.com

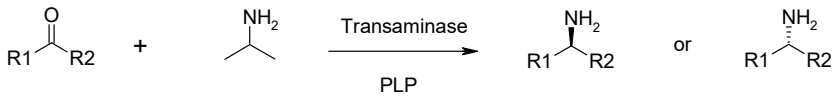
Transaminases and Amine Dehydrogenases SEQENZYM kit

Technical Data Sheet

GENERAL INFORMATION

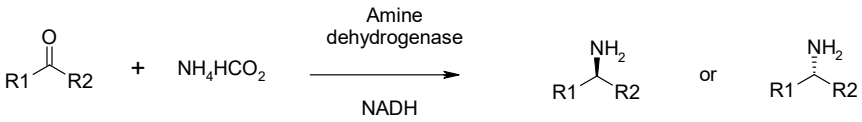
The SEQENZYM™ - Transaminases and Amine Dehydrogenases Kit contains transaminases and amine dehydrogenases that have been selected for their selectivity and for their wide range of potential substrates.

Transaminases catalyze the transfer of an amino group from an amine donor to an amine acceptor, the target substrate. The substrate is generally a prochiral ketone that leads to a chiral amine according to the following scheme.



This transformation depends on the cofactor PLP which is used in catalytic amount and *iso*propylamine is used as the amine donor.

The amine dehydrogenases also catalyze the enantioselective amination of ketones but according to a different mechanism. Firstly, the ketone is converted into the imine in the presence of an ammonium salt. Secondly, the imine is reduced at the expense of NADH into the corresponding chiral amine. Efficient cofactors recycling procedures have been developed by Protéus, please contact us for additional information.



KIT DESCRIPTION

The kit contains 9 transaminases and 3 amine dehydrogenases as cell-free extracts for R&D use only (E4297, E4298, E4300, E4411, E4412, E4413, E4414, E4415, E4416, E4419, E4338, E4339, 50 mg each). Cofactors PLP (Pyridoxal 5'-phosphate, CAS 41468-25-1, 30 mg) and NADH (β-Nicotinamide adenine dinucleotide, reduced disodium salt, CAS 606-68-8, 245 mg) are provided. The screening kit contains sufficient enzyme and cofactors to perform 5 assays per enzyme at 1 mL scale. Buffer salts, DMSO and *i*PrNH$_2$.HCl are not provided; for buffer preparation, see “ADDITIONAL INFORMATION”.

70, allée Graham Bell - Parc Georges Besse - 30035 Nîmes cedex 1 - France - Tél.: +33(0)466 70 64 64 - Fax +33(0)466 70 64 60 - seqensrdnimesinfo@seqens.com - www.proteus.seqens.com

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Enzyme	Enzyme type	Selectivity	Cofactor	Buffer
E4297	Transaminase	*R*	PLP	100 mM sodium phosphate pH 8
E4298	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4300	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4411	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4412	Transaminase	*R*	PLP	100 mM sodium phosphate pH 8
E4413	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4414	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4415	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8
E4416	Transaminase	*S*	PLP	100 mM sodium phosphate pH 8

Enzyme	Enzyme type	Selectivity	Cofactor	Buffer
E4419	Amine dehydrogenase	*R*	NADH	1 M ammonium formate, pH 8.5
E4338	Amine dehydrogenase	*R*	NADH	1 M ammonium formate, pH 8.5
E4339	Amine dehydrogenase	*R*	NADH	1 M ammonium formate, pH 8.5

SCREENING PROCEDURE FOR TRANSAMINASES

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 9 transaminases):

	Stock solutions		Volume to add for each assay	Additional info
	Amount	Dissolve in		
PLP	5 mg	1.5 mL of 100 mM sodium phosphate pH 8	150 µL	-
***i*PrNH$_2$.HCl**	354 mg	3.7 mL of 100 mM sodium phosphate pH 8	400 µL	Adjust the pH to 8 with NaOH 2 N
Substrate	0.20 mmol	500 µL of DMSO	50 µL	-
Transaminase	10 mg	400 µL of 100 mM sodium phosphate pH 8	400 µL	Homogeneize well with pipet*

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

2. In a vial, mix 150 µL of PLP solution, 400 µL of *i*PrNH$_2$ and 50 µL of substrate.

3. Start the reaction with the addition of 400 µL of the suspension of transaminase. Heat the reaction mixture at 30 °C under magnetic agitation.

4. After approximately 24 hours, analyze the reaction by any preferred method to determine the conversion of the ketone to the target amine and the enantiomeric excess.

SCREENING PROCEDURE FOR AMINE DEHYDROGENASES

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 3 amine dehydrogenases):

	Stock solutions		**Volume to add for each assay**
	Amount	Dissolve in	
NADH	47 mg	1.5 mL of 1 M ammonium formate pH 8.5	450 µL
Substrate	0.10 mmol	250 µL of DMSO	50 µL
Amine Dehydrogenase*	10 mg	500 µL of 1 M ammonium formate pH 8.5	500 µL

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

2. In a vial, mix 450 µL of NADH solution and 50 µL of substrate.

3. Start the reaction with the addition of 500 µL of the suspension of amine dehydrogenase. Heat the reaction mixture at 30 °C under magnetic agitation.

4. After approximately 24 hours, analyze the reaction by any preferred method to determine the conversion of the ketone to the target amine and the enantiomeric excess.

STORAGE

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

Prepare freshly cofactor and enzyme solutions before use.

ADDITIONAL INFORMATION

Preparation of 100 mM sodium phosphate pH 8 buffer:

Mix 1.323 g of Na_2HPO_4 and 82 mg of NaH_2PO_4, dissolve in H_2O and complete to 100 mL.

Preparation of 1 M ammonium formate pH 8.5 buffer:

Dissolve $HCOONH_4$ (3.16 g, 50.1 mmol) in water (45 mL) then add NH_4OH (28% NH_3 in H_2O) to adjust the pH to 8.5.

AFTER YOUR FIRST TRIALS,

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http://www.proteus.seqens.com

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