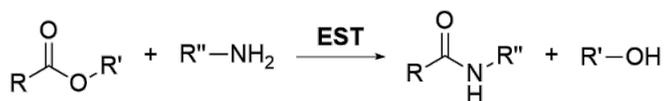


Reactions of Interest

Amidation



Hydrolysis



Codex[®] Esterase Kit General Information

- The Codex[®] Esterase Screening Kit is a useful tool to quickly determine the feasibility of using esterases for amidation or ester hydrolysis reactions. The 12 enzymes included in this kit were developed using Codexis' CodeEvolver[®] technology platform and have been engineered for enhanced activity, substrate range, and solvent and temperature stability.
- The recommended storage temperature for the enzyme powders is -20 °C.
- The Codex[®] Esterase Screening Kit contains sufficient enzyme (250 mg each) for at least 20 screens using the protocol given. Alternatively, fewer screens can be performed, and the remaining enzyme can be used for confirmation and optimization reactions.
- Gram quantities of the esterases in this kit are available from stock for any follow up work, and kilogram to MT quantities can readily be manufactured upon request for development and commercialization.

Screening Reagents Required

- Codex[®] Esterase Kit enzyme powders are provided at 250 mg per enzyme.
- Ester substrate to be provided by customer (at least 1.5 mmoles recommended per screen).
- Amine substrate is not provided (for amidation reaction only, at least 2 mmoles recommended per screen), • Triethanolamine-HCl buffer, 0.25 M, pH 8, to be provided by customer (at least 10 mL recommended per screen).
- DMSO to be provided by customer (at least 1 mL recommended per screen).
- Acetonitrile or extraction solvent to be provided by customer (20 mL recommended per screen).

General Screening Pointers

- Depending on the complexity of your substrate, the enzyme loading, and reaction time can be significantly reduced from the protocol given.
- Some esters can be easily absorbed into common laboratory plastic vials and plates. If you notice the disappearance of the ester substrate in the buffer control, it may be helpful to switch to glass vials.
- Other buffers besides triethanolamine can be used, however, some buffers, such as sodium phosphate, at high concentrations will phase separate with acetonitrile. It is recommended to use a different buffer or quenching solvent if this is the case.
- It may be necessary to perform the amidation reaction in a non-aqueous solvent to achieve high amide yields. Recommended solvents include alkanes such as isooctane or heptane, THF, MTBE and others (ester solvents should be avoided). The free amine should be used in these reactions. Water content of the solvents can have a significant effect on activity. Dried solvent can be mixed with different ratios of water saturated solvent to measure this effect.

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Screening Protocol

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Screening Procedure for Ester Hydrolysis or Ester Amidation

- Allow the enzyme powders to acclimate to room temperature for ~30 minutes.
- Setup the assay for either the hydrolysis or the amidation reaction per the table below:

Step	Hydrolysis	Amidation
① Esterase Preparation	<ul style="list-style-type: none">• Weigh out ~10 mg of each esterase 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 Preparation of Ester Solution	Dissolve the ester of interest in DMSO to give 1.5 M. Prepare at least 0.75 mL of solution.	
③ Preparation of Amine Solution	Not applicable.	Dissolve the amine of interest in 0.25 M triethanolamine buffer, pH 8 to give 0.25 M. If necessary, titrate with HCl until the final pH is 8 (alternatively, the HCl salt, if available, can be used). Prepare at least 7 mL of this solution.
④ Reaction Initiation	<ol style="list-style-type: none">1. Add 450 µL of 0.25 M triethanolamine buffer, pH 8 to each vial containing the esterase powder and an additional vial that does not contain any esterase (negative control).2. Add 50 µL of the ester solution in DMSO to each vial.	<ol style="list-style-type: none">1. Add 450 µL of the 0.25 M amine solution made in step 3 to each vial containing the esterase powder and an additional vial that does not contain any esterase (negative control).2. Add 50 µL of the ester solution in DMSO to each vial.
⑤ Overnight Incubation	Incubate the reactions for ~24 hours at 40 °C with agitation. This 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	Reverse phase HPLC: <ul style="list-style-type: none">• Add 1.5 mL of acetonitrile to each vial. Close vial and mix well.• Centrifuge or filter vial contents to remove any precipitated solids.• Remove an aliquot and analyze by reverse phase HPLC. Normal phase HPLC or GC: <ul style="list-style-type: none">• Extract reaction into 1 mL of extraction solvent (ethyl acetate, MTBE, etc.). If quantifying the acid is of interest, acidify the reaction before extracting. The acidification conditions should be kept mild to avoid any non-selective ester/amide hydrolysis.• Remove an aliquot and analyze by normal phase HPLC or GC.	
Final Reaction Conditions	<ul style="list-style-type: none">• Esterase powder: 20 g/L• Ester: 0.15 M• DMSO: 10 v/v%• Buffer: 0.225 M triethanolamine, pH 8	<ul style="list-style-type: none">• Esterase powder: 20 g/L• Ester: 0.15 M• Amine: 0.225 M• DMSO: 10 v/v%• Buffer: 0.225 M triethanolamine, pH 8

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Screening Protocol

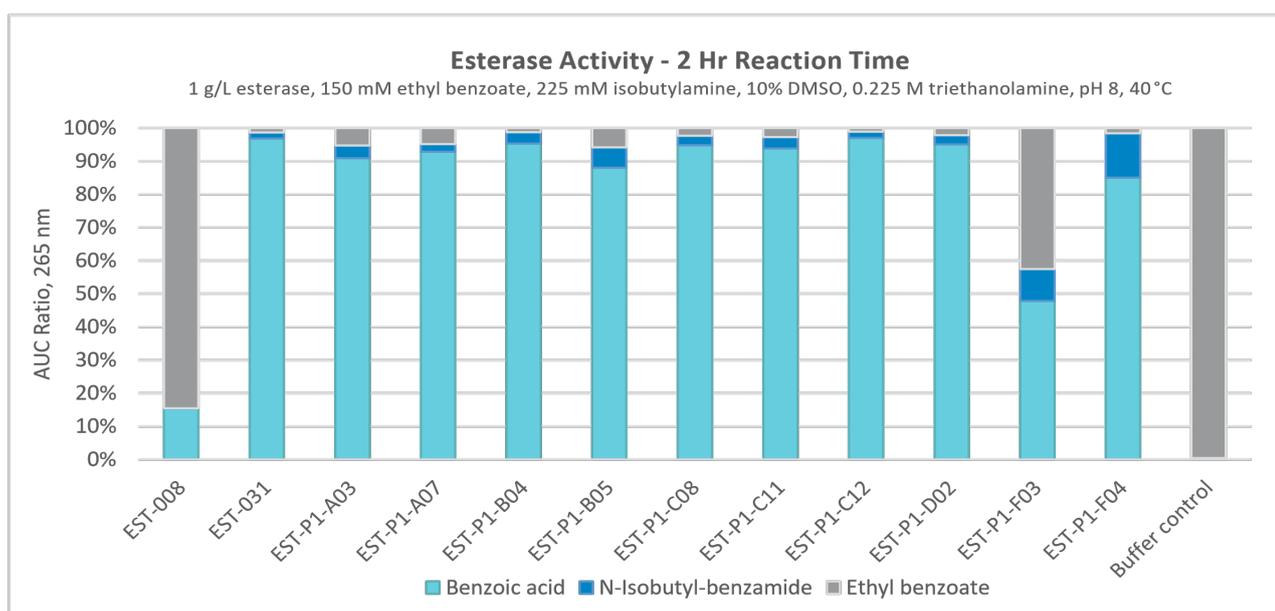
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Positive Control Reaction

If desired, a positive control reaction can be performed for the amidation reaction using isobutylamine hydrochloride (TCI catalog #I0096 or similar) and ethyl benzoate (Sigma catalog #E12497 or similar). The analytical method provided below can be used.

Parameter	Details
Column:	Agilent Eclipse XDB C18 Column (4.6 x 150 mm, 5 µm particle size, catalog #993967-902)
Column temperature:	50°C
Mobile phases:	A: H ₂ O + 0.1% TFA B: MeCN + 0.1% TFA
Mobile phase gradient:	0 – 0.25 min: hold at 30% B 0.25 – 1.0 min: ramp to 95% B 1.0 – 1.5 min: hold at 95% B 1.5 – 1.51 min: ramp to 30% B 1.51 – 2.25: hold at 30% B
Total run time:	2.25 min
Mobile phase flow rate:	2 mL/min
Injection volume:	5 µL
Detection wavelength:	260 nm
Retention times:	Benzoic acid: 1.25 min (hydrolysis byproduct) N-Isobutyl-benzamide: 1.6 min (amidation product) Ethyl benzoate: 1.85 min (substrate)

Expected results (Note: Activity is high on this substrate pair, therefore, the enzyme loading and reaction times were reduced.)



For further information, please contact us at: sales@codexis.com.

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Screening Protocol

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Positive Control Reaction

Enzymes in the Esterase Screening Kit show varying activities across the following substrates that were tested in the hydrolytic reaction direction:

