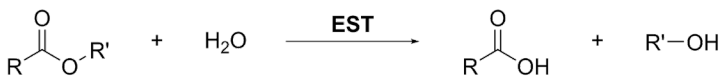
Codex® Esterase Screening Panel

Screening Protocol

REACTIONS OF INTEREST

Amidation

Hydrolysis



CODEX® ESTERASE PANEL GENERAL INFORMATION

- The Codex® Esterase panel is a useful tool to quickly determine the feasibility of using esterases for amidation or ester hydrolysis reactions. The panel contains enzymes that have been selected for their broad substrate range for the synthesis of amides from the corresponding ester and amine substrates.
- The enzymes included in this panel were developed using our CodeEvolver® technology platform and have been engineered for enhanced activity, substrate range, and solvent and temperature stability.
- The Codex® Esterase panel is a 96-well plate containing 100 µL enzyme lysate in each well.
- Recommended storage temperature is -20 °C when stored for up to 6 months, and -80 °C when stored for longer periods.

SCREENING REAGENTS REQUIRED

- Codex® Esterase enzyme lysate in panel, provided.
- Ester substrate, not provided.
- Amine substrate, not provided (for amidation reaction only).
- Triethanolamine buffer, pH 7.5, not provided (for amidation reaction only).
- Acetonitrile, not provided.
- Potassium phosphate buffer, pH 7.5, not provided (for hydrolysis reaction only).
- DMSO, not provided (for hydrolysis reaction only).

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SCREENING PROCEDURE FOR AMIDATION AND ESTER HYDROLYSIS

1. Thaw the 96-well plate at room temperature for ~30 minutes.
2. Optional: Centrifuge the thawed 96-well plate at 4000 RPM (3,220 x g) for 5 minutes at 4 °C.
3. Setup the assay for either the amidation or the hydrolysis reaction per the table below for a total reaction volume of 220 µL. The amidation reaction should have a final pH between pH 8-9.

Reagent concentration for amidation reaction	Volume per well	Volume per plate	Final concentration
Enzyme lysate	100 µL	5.6 mL	45.5% v/v
Amine substrate 900 mM amine in 200 mM TEoA, buffer, pH7.5	75 µL	7.2 mL	34% v/v 307 mM amine 68 mM TEoA
Ester substrate 1M ester substrate in neat acetonitrile	45 µL	4.32 mL	20.5% v/v 205 mM ester ~20% acetonitrile
Total volume	**220 µL**	**21.12 mL**	

Reagent concentration for ester hydrolysis reaction	Volume per well	Volume per plate	Final concentration
Enzyme lysate	100 µL	9.6 mL	45.5% v/v
Buffer 275 mM Potassium phosphate buffer, pH 7.5	80 µL	7.68 mL	36.4% v/v 100 mM buffer
Ester substrate 27.5-55 mg/mL ester substrate in DMSO	40 µL	3.84 mL	18.1% v/v 5-10 mg/mL ester ~18.1% DMSO
Total volume	**220 µL**	**21.12 mL**	

4. Seal each plate (when using heat-sealer, set at 180 °C for 3 seconds).
5. Quickly spin the reactions to remove residual droplets from the side of the wells.
6. Run the reaction overnight (16-22 hours) while shaking at 50 °C for amidation and 30 °C for ester hydrolysis.

CODEX® ESTERASE WORK-UP AND ANALYSIS FOR AMIDATION AND ESTER HYDROLYSIS

1. Quickly centrifuge the reactions to remove residual droplets from the plate seal prior to removal.
2. Add 660 µL of acetonitrile per well to quench the reaction. Re-seal plate and shake for 15 minutes at room temperature.
3. Centrifuge the plate at 4,000 rpm for 10 minutes to precipitate the proteins.
4. Transfer 200 µL of the clear supernatant from each well to a shallow 96-well plate. Alternatively, the samples can be removed from the plate and transferred to HPLC vials.
5. Seal the shallow well plate and analyze the samples using a preferred HPLC/MS method to determine if the desired product was produced.

USEFUL TIPS

- If testing the feasibility of the reaction, start by using a high enzyme loading, low to moderate substrate loading, and long reaction time to increase the chance of identifying an active biocatalyst.
- If testing the feasibility of a process, test variants under desired process conditions.
- The best hits can be verified using lyophilized powders of the individual enzymes, which can be custom-made by Codexis. When using lyophilized powders amidation reactions can be tested with other solvents such as toluene and very little water to minimize undesired hydrolysis.
- By sharing the conversion and selectivity results from your screen with Codexis, we can provide suggestions for follow-up enzyme libraries.

ANALYTICAL METHOD FOR THE ISOBUTYLBENZAMIDE PRODUCT FROM COUPLING ISOBUTYLAMINE AND ETHYL BENZOATE

Injection Volume:	10 µL
Column:	Agilent Eclipse XDB C18 Column (4.6 x 150 mm, 5 µm particle size)
Mobile Phases:	A: H2O; B: 0.1% trifluoroacetic acid in acetonitrile
Gradient:	30% B, 2.75 min – 95% B, 3.75 min – 95% B, 3.90 min – 30%
Total Run Time:	5.5 min
Flow Rate:	1.5 mL/min
Retention Times:	2.9 min (isobutylbenzamide); 3.7 min (ethylbenzoate); 2.4 min (benzoic acid, side product)
Wavelength:	260 nm

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

CODEXIS®

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