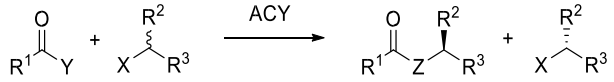
Codex® ACY Panel

Screening Protocol

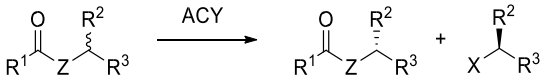
Reactions of Interest

Condensation



X = OH, NH_2
Y=OH, NH_2, OMe
Z=O, NH
R^1= $-CH_2Ar$, $-CH_2OAr$, -CH(Y)Ar

Resolution/Hydrolysis



X=OH, NH_2
Z=O, NH
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Codex® ACY Panel Plate Information

- The Codex® ACY Panel is a collection of Acylase enzymes that are capable of ester formation/transesterification or ester hydrolysis. Some of the enzymes included in this panel were developed using Codexis' CodeEvolver® protein engineering technology and have been engineered for enhanced activity, substrate range, and solvent and temperature stability
- The Codex® ACY Screening Plate consists of one 96-well plate that contains 100 µL enzyme solution (lysate) in each well. This pre-arrayed 96-well panel presents an easy-to-use and convenient format for screening. Negative controls are present in wells E1-E4 and PGA-000 in wells E5-E8 as a positive control.
- Recommended storage temperature for the plates is-20 °C

Required screening reagents

- The Codex® ACY-Panel, supplied in 96-well plate format
- 50 mM potassium phosphate buffer, pH 7.0 or MTBE (not provided)
- Your substrate(s) of interest

Screening Procedure

Setup the assay for either condensation or resolution/hydrolysis reaction per the table below:

Step	Condensation	Resolution/Hydrolysis
1. Plate Preparation	Thaw the Codex® ACY Panel Screening Plate at room temperature for approx. 30 min. Store plate at 4 °C until use; thaw plate only on the day of use.	
2. Preparation of Substrate(s) Solution	Make 25 mL 1.5x substrate stock solution in MTBE. The solution should have 15 mM acid and 30 mM amine or alcohol.	Make 25 mL 15 mM (1.5x) substrate stock solution in 50 M potassium phosphate buffer, pH 7 or MTBE. If using buffer, pH the solution back to 7.0.
3. Reaction Initiation	a. Unseal the plate. b. Add 200 µL substrate(s) solution from step 2 to each well. c. Seal the assay plate (180 °C, 3 sec if using a heat seal).	
4. Overnight Incubation	Shake the plates on titer plate shaker (low speed) at room temperature for 24 hours.	
5. Reaction Workup and Analysis	Reverse phase HPLC: • Add 0.3 mL of acetonitrile to each vial. Reseal and mix well. More acetonitrile can be added if one volume is not enough to solubilize the substrate(s) and product. • Centrifuge plate at ~4000 RPM for 10 minutes to precipitate any solids. • Remove an aliquot from each well and analyze by reverse phase HPLC. Normal phase HPLC or GC: • Add 50 µL 6 N HCl and 1 mL MTBE to each well. Reseal and mix well • Centrifuge plate at ~4000 RPM for 2 minutes to separate the phases. If a centrifuge is not available, the phases can be separated unaided upon sitting. • Remove an aliquot from each well and analyze by normal phase HPLC or GC.	
6. Final Reaction Conditions	• Lysate volume: 100 µL/well (33% v/v) • Acid: 10 mM • Amine or alcohol: 20 mM • MTBE: 200 µL /well Total volume: 300 µL /well	• Lysate volume: 100 µL/well (33% v/v) • Substrate: 10 mM • MTBE or potassium phosphate buffer: 200 µL /well Total volume: 300 µL /well

For any additional support, please contact us at sales@codexis.com