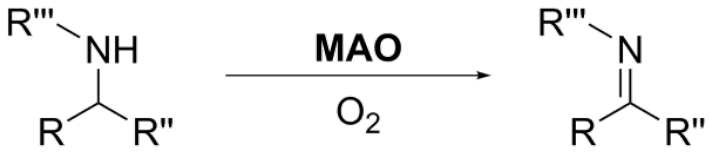
Codex® MAO Panel

Screening Protocol

Reactions of Interest



Codex® MAO Screening Panel General Information

- The Codex® MAO Panel is a 96-well plate containing 100 µL Monoamine Oxidase (MAO) enzyme lysate in each well. The panel is a great tool for feasibility assessment of using MAOs for the conversion of amine to imines (asymmetric or resolution) using ambient oxygen as an oxidant.
- The enzymes included in this kit were developed using Codexis' CodeEvolver® protein engineering technology and have been engineered for improved activity and stability for the conversion of amines to imines.
- Recommended storage temperature for the plates is-20 °C.

Screening Reagents Required

- Codex® MAO enzyme lysate in panel, provided.
- Catalase (CAT-101), 100U, provided.
- 100 mM potassium phosphate buffer, pH 7.5, not provided.

Codex® MAO Screening Panel Procedure

1. Defrost plates at 4 °C. Centrifuge the thawed plate at 4000 rpm (3,220 x g) for 2 minutes at 4 °C.
2. Setup the assay per the table below for a final reaction volume of 200 µL per well.

Reagent	Volume per Well	Volume per Plate	Final Concentration
Lysate	100 µL	9.60 mL	33% v/v
100 mM substrate in buffer	50 µL	4.8 mL	25% v/v 25 mM amine
20 U/mL Catalase[1] in buffer	50 µL	4.8 mL	25% v/v 5 U/mL Catalase
Total Volume	200 µL	19.2 mL	25 mM amine

[1] Catalase from Aspergillus niger (Sigma-Aldrich C3515) or from Novozymes has been successfully used in numerous experiments.

3. Assay setup:
   - Unseal the plates.
   - Add the substrate solution to each well.
   - Add the catalase solution to each well.
   - Seal the assay plate (when using heat-sealer, set at 180 °C for 3 seconds). Quickly spin the reactions to remove residual droplets from the side of the wells.
4. Shake the plates on titer plate shaker at low speed and at room temperature for 24 hours.

Codex® MAO Panel Work Up and Analysis

1. Reaction quenching:
   a. Quickly centrifuge the assay plates (4000 rpm, 1 minute at 4 °C) to remove residual droplets.
   b. Unseal the assay plates.
   c. Quench the reaction with either of the following methods:
      i. Add 1000 µL of quenching solvent (e.g. acetonitrile, methanol or MTBE) to each well.
      ii. Add 100 µL of 6 N NaOH to each well, followed by 0.5-1 ml of MTBE to each well.
   d. Re-seal the quenched plates (when using heat-sealer, set at 180 °C for 3).

2. Shake the plates on titer plate shaker (medium-high speed) at room temperature for 20 minutes.

3. HPLC sample preparation:
   a. Centrifuge the assay plates (4000 rpm, 20 minutes, 4 °C).
   b. Unseal the quenched plates.
   c. Transfer 200 µL of samples (or the organic layer) from quenched plates to round-bottom shallow well plates.
   d. Seal the shallow well plates (when using heat-sealer, set at 180 °C, 2 sec).

4. Analyze using HPLC method of choice to determine if the desired product was produced.

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