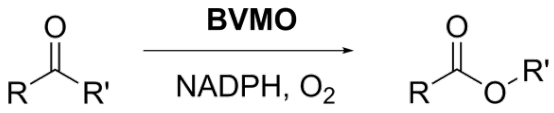
Codex® BVMO Panel

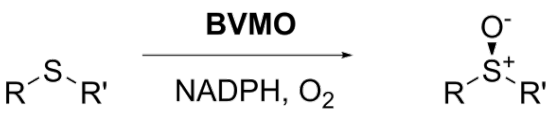
Screening Protocol

Reactions of Interest

Bayer-Villiger Oxidation



Sulfoxidation



Codex® BVMO Screening Kit General Information

- The Codex® BVMO Panel is a collection of Baeyer-Villiger Monooxygenases (BVMOs) in 96-well plates containing 100 µL BVMO lysate in each well. BVMOs have been developed for the asymmetric oxidation of sulfide to sulfoxide. The enzymes included in this panel were developed using Codexis' CodeEvolver® protein engineering technology and have been engineered for improved activity and stability.

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

Screening Reagents Required

- Codex® BVMO Panel. Two 96-well plates, provided.
- 200 mg recycling KRED, provided.
- 100 mg NADP+, provided.
- 200 mg GDH-105, provided.
- 50 or 100 mM potassium phosphate buffer, pH 9.0, not provided.
- Isopropanol or glucose, not provided.

Note: If the substrate contains a ketone or aldehyde function that reacts with the KRED, then consider replacing the KRED recycling system with GDH/glucose recycling system.

Codex® BVMO Screening Procedure

1. Defrost plates, which contain 100 µL lysate per well, at 4 °C. Centrifuge (4000 rpm, 2 min, 4 °C).

2. Setup the assay using either the KRED/IPA or the GDH/glucose cofactor recycling system per the table below for a final reaction volume of 300 µL per well.

Reagent concentration using KRED/IPA for cofactor recycling	Volume per well	Volume per plate	Final concentration
Lysate	100 µL	9.60 mL	33% v/v
Solution A 1.8 g/L KRED-P1-A12 0.9 g/L NADP+ 50 mM buffer	170 µL	16.32 mL	57% v/v 1 g/L KRED-P1-A12 0.5 g/L $NADP^+$
Solution B 10–50 mg/mL substrate in Isopropanol	30 µL	2.88 mL	10% v/v 1–5 mg/mL substrate 10% isopropanol
Total Volume	300 µL	28.8 mL	

Reagent concentration using GDH/glucose for cofactor recycling	Volume per well	Volume per plate	Final concentration
Lysate	100 µL	9.60 mL	33% v/v
Solution A 1.8 g/L GDH-105 0.9 g/L $NADP^+$ 100 mM buffer	170 µL	16.32 mL	57% v/v 1 g/L GDH-105 0.5 g/L $NADP^+$
Solution B 10–50 mg/mL substrate in buffer or co-solvent 15–75 mg/mL glucose	30 µL	2.88 mL	10% v/v 1–5 mg/mL substrate 1.5–7.5 mg/mL glucose
Total Volume	300 µL	28.80 mL	

3. Assay setup:
   a. Unseal the plates.
   b. Add 170 µL of Solution A to each well.
   c. Add 30 µL of Solution B to each well.
   d. Seal the assay plate using a plate sealer (180 °C, 3 sec).

4. Shake the plates on titer plate shaker at low speed and at room temperature for 24 hours.

Codex® BVMO Panel Work Up and Analysis

1. Reaction quenching:
   a. Centrifuge the assay plates (4000 rpm, 1 min, 4 °C).
   b. Unseal the assay plates.
   c. Add 1000 µL of quenching solvent (e.g. acetonitrile, methanol, or MTBE) to each well.
   d. Seal the quenched plates (180 °C, 3 sec).

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 min, 4 °C).
   b. Unseal the quenched plates.
   c. Transfer 200 µL of samples from quenched plates to round-bottom shallow well plates (Corning 3365).
   d. Seal the shallow well plates (180 °C, 2 sec).

4. Analyze using HPLC method of choice.

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

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