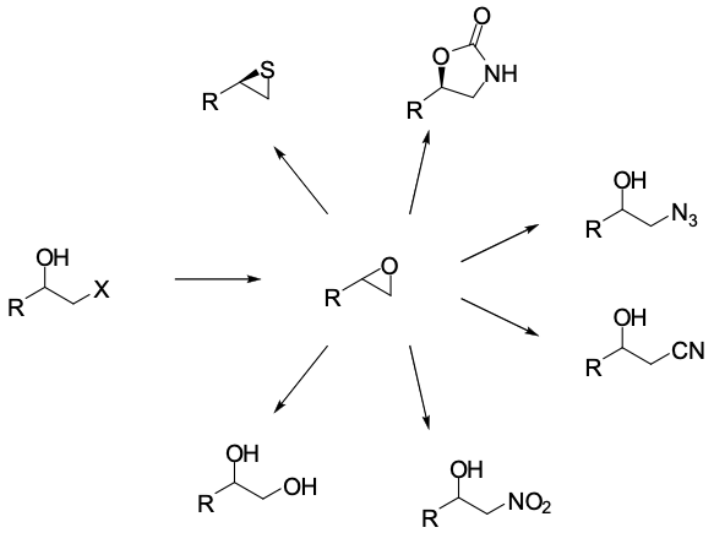
Codex®
HHDH Panel

Screening Protocol

Document # PRO-015-03

Reactions of Interest



Codex® HHDH Panel Plate Information

- Recommended storage temperature for the plates is -20 °C.
- The plates are from Corning (product number 3961) and can be purchased from VWR (catalog number 29445-166).

Codex® HHDH Panel Materials and Reagents

- Two 96-well plates, provided.
- DMSO, not provided.
- 100 mM Tris-SO4 buffer, pH 7, not provided.

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Codex® HHDH Panel

Codex® HHDH Panel Screening Procedure

1. Defrost plates, which contain 100 µL lysate per well, at 4 °C. Centrifuge (4000 rpm, 2 min, 4 °C).
2. Assay setup (final reaction volume 300 µL per well):
   a. Unseal the plates.
   b. Add the substrate solution to each well.
   c. Seal the assay plate (180 °C, 3 sec).
3. Shake the plates on titer plate shaker (low speed) at room temperature for 24 hours.

Reagent Concentration for Epoxide formation	Volume per well	Volume per plate	Final concentration
Lysate	100 µL	9.60 mL	40% v/v
Substrate solution 75 mM halohydrin 8.3% DMSO Buffer	150 µL	14.4 mL	60% v/v 45 mM halohydrin 5% DMSO Buffer
Total Volume	250 µL	24.0 mL	

Reagent Concentration for Epoxide formation	Volume per well	Volume per plate	Final concentration
Lysate	100 µL	9.60 mL	40% v/v
Substrate solution 10 mM epoxide 20 mM nucleophile Up to 8.3% DMSO Buffer	150 µL	14.4 mL	60% v/v 10 mM epoxide 20 mM nucleophile Up to 5% DMSO Buffer
Total Volume	250 µL	24.0 mL	

Codex® HHDH Panel

Codex® HHDH 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).
4. Seal the shallow well plates (180 °C, 2 sec). Analyze using HPLC method of choice.

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

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