

# COPOX Activity Assay

## COPROPORPHYRINOGEN III OXIDASE (COPOX) ASSAY

### Reaction A: Uroporphyrinogen III synthesis

1. Add 8  $\mu\text{L}$  0.5  $\mu\text{g}/\mu\text{L}$  rPBGD (recombinant porphobilinogen deaminase) stock and 2  $\mu\text{L}$  1  $\mu\text{g}/\mu\text{L}$  rU3S (recombinant uroporphyrinogen III synthase) to 70  $\mu\text{L}$  10mM dithiothreitol in 0.1M Tris pH 7.65.
2. Perform subsequent steps in the dark until the addition of HCl.
3. Start the synthesis of substrate by adding 15  $\mu\text{L}$  2.2mM PBG (prophobilinogen) in 0.1M Tris pH7.65.
4. Incubate the mixture in a 37°C water bath for 35 min.
5. Neutralize the reaction mixture with 20  $\mu\text{L}$  0.15M  $\text{KH}_2\text{PO}_4$  and cool in an ice bath for at least 2 min.

### Reaction B: Coproporphyrinogen III synthesis

1. Add 75  $\mu\text{L}$  of a mixture containing 10  $\mu\text{g}$  rhUroD (recombinant human uroporphyrinogen deaminase) and 8  $\mu\text{g}$  bovine serum albumin in 50mM KPi (potassium phosphate) pH6.8 to Reaction A.
2. Incubate at 37°C for 1h in the dark.
3. Adjust the pH to 7.5-8.0 with about 0.3  $\mu\text{L}$  4M KOH.

### Reaction C: COPOx assay

1. Add 50  $\mu\text{L}$  of 0.2  $\mu\text{g}$  protein/ $\mu\text{L}$  sample in homogenization buffer to Reaction B.
2. Incubate at 37°C water bath for 30 min.
3. Add 80  $\mu\text{L}$  6M HCl to stop the reaction.
4. Complete the oxidation of all porphyrinogens to porphyrins by exposing the mixture to longwave UV (320-400nm) for 30 min or under bright fluorescent light for 2h.
5. Centrifuge at about 16000xg in regular microfuge for 10 min.
6. Quantify the porphyrins by UPLC (ultra performance liquid chromatography).

Use solutions of known concentrations of authentic porphyrins dissolved in 1.5M HCl as standards (Porphyrin Acids, Chromatographic Marker, Product # CMK-1A, Frontier Scientific,

Logan, Utah). Make similar solutions of protoporphyrin IX for standard quantitation.

Coproporphyringen III substrate may also be chemically synthesized from coproporphyrin III in the presence of palladium carbon and hydrogen gas.

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