COPROPORPHYRINOGEN III OXIDASE (COPOX) ASSAY

Reaction A: Uroporphyrinogen III synthesis

Add 8 µL 0.5 µg/µL rPBGD (recombinant porphobilinogen deaminase) stock and 2mL 1µg/µL rU3S (recombiniant uroporphyrinogen III synthase) to 70µL 10mM dithiothreitol in 0.1M Tris pH 7.65.
Perform subsequent steps in the dark until the addition of HCl.
Start the synthesis of substrate by adding 15 µL 2.2mM PBG (prophobilinogen) in 0.1M Tris pH7.65.
Incubate the mixture in a 37˚C water bath for 35 min.
Neutralize the reaction mixture with 20µL 0.15M KH_2PO_4 and cool in an ice bath for at least 2 min.

Reaction B: Coproporphyrinogen III synthesis

Add 75µL of a mixture containing 10µg rhUroD (recombinant human uroporphyrinogen deaminase) and 8µg bovine serum albumin in 50mM KPi (potassium phosphate) pH6.8 to Reaction A.
Incubate at 37˚C for 1h in the dark.
Adjust the pH to 7.5-8.0 with about 0.3µL 4M KOH.

Reaction C: COPOx assay

Add 50µL of 0.2µg protein/µL sample in homogenization buffer to Reaction B.
Incubate at 37˚C water bath for 30 min.
Add 80µL 6M HCl to stop the reaction.
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.
Centrifuge at about 16000xg in regular microfuge for 10 min.
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.