UROD Activity Assay

UROPORPHYRINOGEN DEAMINASE (UROD) ASSAY

Reaction A: Uroporphyrinogen (substrate) synthesis

1. Add 10 µL 0.5 µg/µL rPBGD (recombinant porphobilinogen deaminase) stock to 75µL 10mM DTT (dithiothreitol) in 0.1M Tris pH 7.65. (This is for the synthesis of uroporphyrinogen I. To make uroporphyrinogen III instead, replace 1.5µL of the Tris/DTT with 1.5µL of 1µg/µL recombinant uroporphyrinogen III synthase or rU3S.)
2. Perform subsequent steps in the dark until addition of HCl.
3. Start the synthesis of substrate by adding 15 µL 2.2mM PBG or porphobilinogen (0.54mg/mL) in 0.1M Tris pH7.65.
4. Incubate the mixture in a 37˚C water bath for 35 min. (Enough rPBGD activity must have been present in the substrate synthesis step above to supply at least 30µM uroporphyrinogen in this 200µL activity assay.)
5. Neutralize the reaction mixture with 20µL 0.15M KH_2PO_4 and cool in ice-bath for at least 2 min.

Reaction B: UROD Assay

1. Add 80µL ice-cold sample to the 120µL substrate (reaction A) solution.
2. Incubate the assay mixture in 37˚C in water bath for 30 min.
3. Add the same volume (200µL) of 3M 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).

> For the blanks and background porphyrins, replace the substrate solution with just the Tris/DTT buffer.

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