U3S Activity Assay

UROPORPHYRINOGEN III SYNTHASE (U3S)

Cytosolic U3S Assay

1. Mix 100 µL 20mM Tris pH 8.2, 260 µL 20mM KPi (potassium phosphate) pH 8.2 and 20 µL 0.5mg/mL rPBGD (recombinant porphobilinogen deaminase).
2. Mix well and incubate for at least 2 min at 37˚C.
3. Add 20µL 2.2mM PBG (porphobilinogen) and incubate 120±2 sec at 37˚C.
4. Add 20 µL sample previously diluted to 2 mg protein/mL with 20mM KPi pH 8.2. (Use 2mg/mL BSA or bovine serum albumin as the zero blank.)
5. Incubate for 120±2 sec at 37˚C in the dark.
6. Stop the reaction with 140 µL 6M HCL.
7. Expose the mixture to UV light for 30 min or ambient light for 2h to oxidize all porphyrinogen formed.
8. Centrifuge at 16000xg for 10 min. Collect the supernatant.
9. Quantify the porphyrins by UPLC (ultra performance liquid chromatography).

Replicates

1. Perform three reactions for each sample, a and b containing PBG, c with just the KPi buffer.
2. For each sample tube a,b,c - reagents were added at 30-sec staggered intervals, hence the 120±2 sec after the first addition of PBG.

For rhU3S protein

1. dilute U3S stock to 0.5 mg/mL with buffer consisting of 10mm Tris pH7.5, 150mM NaCl, 10% glycerol and 1mM dithiothreitol.
2. Use 2 µL of diluted stock in the assay above and 278µL KPi, not 260 µL.

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