ALAD Activity Assay

AMINOLEVULINIC ACID DEHYRATASE (ALAD)

Mouse liver homogenate

1. Weigh out ~100mg liver.
2. Add 400 µL 1mM DTT (dithiothreitol) in 50mM KPi (potassium phosphate) pH 6.8.
3. Homogenize with ten up-and-down strokes in a 2-mL glass-Teflon (Potter- Elvehjem) tissue homogenizer in an ice bath.
4. Store the resulting 20%w/v homogenate at −80 °C until needed.

Cultured cell homogenate

1. Wash the cells with phosphate buffered saline (PBS) pH 7.4.
2. Resuspend in about three pellet volumes of 50mM KPi pH6.3 for mammalian cells, or 200mM glycine/NaOH pH 9.2 for yeast.
3. For mammalian cells, sonicate (homogenize) while in an ice bath at the lowest practicable power setting for 3 cycles x 5 sec at 50% duty (2.5 sec on, 2.5 sec off).
4. Sonicate yeast cells at the highest power setting that does not cause significant aerosolization of the mixture for 12 5-sec cycles.
5. Store the resulting homogenate at −80 °C until needed.

ALAD Assay

1. Prepare two replicate pairs for each sample, two live and two heat-deactivated for 10 min in boiling water (for use as blank).
2. Prepare similar solutions of PBG (porphobilinogen, ALAD product) for use as standard curve.
3. Add 175µL solution containing 1mM DTT and 1.714mM d-aminolevulinic acid or ALA (for a final 1.5mM in 200µL) to 25µL (500µg protein) sample or standard PBG.
4. Incubate at 37˚C for 30 min.
5. Add 400µL of 100mM Tris pH 7.6 containing 150µM succinylacetone (SA) and 1 mg/mL recombinant porphobilinogen deaminase (PBGD). (The Tris adjusted the system to near pH 7.6 for optimal PBGD activity, while the SA inhibited further ALAD activity.)
6. Incubate at 37˚C for 45 min.

7. Add 200µL of 6 M HCl to stop the reaction.
8. Expose the mixture to UV light for 30 min or ambient light for 2h to oxidize all porphyrinogen formed.
9. Centrifuge at 16000xg for 10 min. Collect the supernatant.
10. Quantify the porphyrins by UPLC (ultra performance liquid chromatography).

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