

ULTIMATE 6-AZAZURACIL SENSITIVITY ASSAY FOR YEAST

Treatment of yeast with 6-Azauracil (6AU) leads to a reduction of intracellular GTP levels. The reduction in GTP levels is not itself lethal, but can block yeast growth when combined with mutations that affect transcriptional elongation. 6AU sensitivity thus can be used as a crude assay to test for mutations that affect transcriptional elongation.

A. PREPARATION OF 6AU PLATES.

1. Each assay requires CSM plates and CSM plates containing 100 µg/ml of 6AU. Purchase 6AU from Sigma. Prepare 50 mg/ml stock solution of 6AU in 1M ammonium hydroxide. Add to CSM/agar before pouring (~55°C).

B. SPOTTING.

The assay requires growing saturated cultures of yeast, counting, and spotting between 50,000 and 80 yeast on both CSM and CSM+6AU plates. For most strains we work with, an OD₆₀₀ of 0.5 is equivalent to ~10,000 viable cells per microlitre (note that this will be different for different strains, and dependent on plating efficiency).

1. Grow 5 ml of yeast at appropriate temperature overnight.
2. Vortex. Dilute sample of yeast 1:20 in TE. Measure OD₆₀₀.
3. Dilute yeast to OD=0.5 in TE (10,000/µl). Sample A.
4. Set up 3 tubes with 160 µl of TE (B-D). And an empty tube labeled 'E'.
5. Prepare a series of 5-fold dilutions. Transfer 40 µl of A into B. Vortex. Transfer 40 µl of B into C. Vortex. Transfer 40 µl of C into D. Vortex. Transfer 40 µl of D into E.
6. Spot 5 µl of each dilution on CSM and CSM+6AU plates in duplicate. Grow at appropriate temperature. Photograph.