Protocol

TD-P Revision 3.0

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Nitrogen Starvation Protocol for Yeast

Introduction

Autophagy, the degradation of cellular components, is a process that is often triggered in cells by nutritional limitations, including nitrogen starvation, and is accompanied by change in protein levels and RNA degradation. One way to detect autophagy in cells is to measure levels of acetaldehyde dehydrogenase (Ald6p), which has been found to decrease during autophagy caused by nitrogen starvation. Here, we describe a protocol to detect autophagy by electrophoresis and transmission electron microscopy in starved bacteria or yeast.

Materials

For SD-N medium:

- 0.17% yeast nitrogen base **without** amino acids or ammonium sulfate
- 2% glucose

For the procedure:

- 0.9% NaCl solution
- PMSF (GoldBio Catalog # P-470)

Storage and Handling

- Store PMSF desiccated at 4°C. Protect from light.
- This product may be shipped on ice and should be stored immediately upon arrival at 4°C.

Method

1. Wash yeast cells with sterile 0.9% NaCl solution.

2. Inoculate into SD-N samples both with and without 1mM PMSF to obtain a final cell density of 10 cells/ml and incubate at 17°C.

3. Remove 1 ml samples from cultures **without PMSF** every hour and centrifuge for 5 minutes at 3,000 rpm, at 4°C.

4. Store pellets at -20°C when not in use.

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5. Use pellets to detect the cytosolic acetaldehyde dehydrogenase (*Ald6p*) by Western Blotting.

6. Collect yeast cells from SD-N with PMSF after 8 hours of incubation by centrifugation and prepare for Transmission electron microscopy.

Associated Products

- PMSF (GoldBio Catalog # P-470)

References

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