Complete Yeast Protein Extraction

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Abstract

This protocol is suitable for rapidly generating extracts of fungal cells for gel electrophoresis or western blots. It was optimised for complete extraction of difficult-to-solubilise proteins. This protocol was originally described in von der Haar 2007.

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Protocol

Step 1.

Prepare the basic solutions:

Extraction Buffer: 0.1 M NaOH, 0.05 M EDTA, 2% SDS

(Note: before use, this buffer is supplemented with 2% 2-mercaptoethanol. With 2-ME the shelf life is several hours, without 2-ME the shelf life of this solution is 1 week).

Caution: *This buffer is strongly alkaline and additional protective equipment (gloves and goggles) should be worn while preparing and handling it.*

Neutralisation Buffer: 4 M acetic acid in water. Neat acetic acid is 11 M in concentration.

Loading buffer: 0.25 M Tris-HCl pH 6.8, 50% glycerol, 0.05% bromophenolblue.

Step 2.

Pre-heat a heating block to 96°C. The extraction works efficiently for most proteins at temperatures of 80°C or above.

Step 3.

Harvest 10^8 yeast cells (about 5 oD_{600} units of logarithmically growing yeast) by centrifuging in a microcentrifuge at max speed for 1 minute. Completely remove the supernatant.

Step 4.

Freshly supplement 1 ml of extraction buffer with 20 µl 2-mercaptoethanol (scale this up for more than 5 extractions). Resuspend the pelleted yeast cells in 200 µl of the supplemented extraction buffer. Incubate the resuspended cells at 96°C fo 10 minutes.

Step 5.

Add 5 µl of neutralisaton buffer, vortex the cells.

Step 6.

Add 50 µl of loading buffer, vortex again.

Step 7.

The extract is now ready to be loaded onto SDS-PAGE gels. Clearing by centrifugation can be done but is not required, and loadig the uncleared (but well resuspended) cell slurry into the pockets of the gel aids extraction.

Warnings

The extraction buffer is strongly alkaline. Additional protective equipment (gloves and goggles) should be worn while handling this buffer.