

Protein extraction from yeast Pichia pastoris with

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CONTEXT

This laboratory develops biomolecular solutions for plant biomass production. The proteins extraction from methylotrophic yeast is a part of the laboratory's daily work.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-004 (0.5 mm glass beads).
- Samples: 5.108 to 1.109 of Pichia pastoris (pellets).
- Buffer: 500 µL of Pichia breaking buffer (pH7.4, 50mM Na-Phosphate, 1mM EDTA, 5%(v/v) glycerol).

PROTOCOL

- Minilys: Two different speeds and different durations of cycle were tested to lyse yeast cells. Duplicate (5000 rpm) or triplicate (4000 rpm) by condition.
- >5000 rpm 20 sec x 2, 4, or 6 with 60 sec break on ice between each cycle.
- >4000 rpm 20 sec x 2 with 60 sec break on ice between each cycle.
- Analysis: SDS-PAGE.

RESULTS

The following treatment 4000 rpm 20 sec x 2 already gave good extraction of protein. There were no sign of the excess disintegration of the proteins even after 5000 rpm 20 sec x 6 treatment. Even the 5000 rpm 20 sec x 6 treatment gave no excess heat and the tube was less than 30°C after each treatment.

5000 rpm, 20 sec 2 cycles 4 cycles 6 cycles

4000 rpm, 20 sec 2 cycles

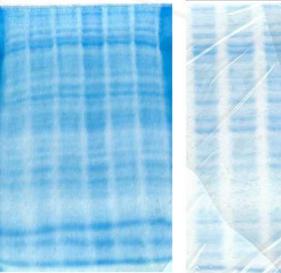


Figure 1: SDS-PAGE of the protein extracted from Plchia pastoris







CONCLUSION

Buffer extraction with 4000 rpm, 20 sec x 2 treatment allows to have enough protein yield for the experiment while further treatment up to 5000 rpm 20 sec x 6 gave no sign of excess disintegration of the extracted proteins.

Minilys's ease of handling and good extraction power provide efficient and crosscontamination free throughput when the number of samples are small.



For more details, please contact precellys@bertin.fr



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