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Yeast competent cells

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Protocol status: Working

We use this protocol and it's working



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Abstract

This is a protocol to make yeast competent cells, which can be then stored at 4 °C for up to a week.

Materials

MATERIALS

 YPD Broth Bio Basic Inc. Catalog #SD7022.SIZE.250g

Troubleshooting



- 1 Pick a yeast colony from a plate and place it inside 10 mL YPD medium.
- 2 Incubate the cells overnight at 30 °C and 180 rpm.
- 3 The next day, measure the OD600 of the liquid culture.
- 4 Inoculate a flask with 50 mL YPD medium with enough volume of the overnight culture to reach an OD600 of 0.4.
- 5 Incubate the cells for 3-4 hours at 30 °C and 180 rpm until an OD600 of 2.0 is reached.
- 6 Place the 50 mL of liquid culture inside a 50 mL tube and centrifuge the cells at 3000x g for 5 minutes and 20 °C.
- 7 Discard the supernatant and resuspend the cells in 25 mL of sterile MQ water. Centrifuge cells at 3000x g for 5 minutes and at 20 °C. Repeat this step another time.
- 8 Discard the supernatant and resuspend the cells in 1 mL sterile MQ water. Transfer the volume into a 1.5 mL tube.
- 9 Centrifuge the cells at 3000x g for 5 minutes and discard the supernatant.
- 10 Resuspend cells in 1 mL sterile MQ water and aliquote them by 100 µL in different 1.5 mL tubes. Each tube can be used for a transformation and may be stored at 4 °C for up to a week.