MB134_SUP4_EN_V2401

NZY Total RNA Isolation Kit

Catalogue number	Presentation
MB13402	50 columns

Support Protocol for the isolation of total RNA from up to 5x10^7 Yeast cells

I. Sample preparation

Two alternative protocols can be used in yeast cells homogenization. Users may choose between an enzymatic digestion **(a)** or mechanical homogenization **(b)**.

This depends on the available laboratory equipment and personal preference. Homogenization by enzymatic digestion is only recommended for fresh harvested cells. On the other hand, homogenization by mechanical disruption can be performed with yeast cell pellets, stored at -70°C for several months.

a) Homogenization by enzymatic digestion

1. Centrifuge YPD culture at 5,000 xg for 10 min and harvest 2–5 mL.

2. Resuspend pellet in an appropriate amount of sorbitol/lyticase buffer (50–100 U lyticase or zymolase in 1 M sorbitol/100mM EDTA) and incubate at 30°C for 30 min.

3. Centrifuge at 1,000 xg for 10 min and carefully discard supernatant. Depending on the yeast strain it may be necessary to optimize incubation time and lyticase/zymolase concentration.

Proceed with step 1 of the NZY Total RNA Isolation Kit protocol.

b) Homogenization by mechanical disruption

1. Centrifuge YPD culture at 5,000 xg for 10 min, harvest 2–5mL and wash with ice-cold water.

2. Mix 350 µL Buffer NR and 3.5 µL ß-mercaptoethanol and resuspend the cell pellet. Add glass beads (e.g., 300 mg glass beads, 425–600 µm). Shake samples in a swing-mill at 30 Hz for 15 min.

Proceed with step 2 of the NZY Total RNA Isolation Kit protocol.

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