Dynabeads® mRNA DIRECT™ Micro Kit

Isolate mRNA for reverse transcription and PCR

Cat. no. 610.21

Initial Check-List

→ All buffers, except the 10 mM Tris-HCl, should be brought to room temperature prior to use. The 10 mM Tris-HCl should be stored on ice or 2-8°C prior to use.

→ Ensure that the Dynabeads® Oligo $(dT)_{25}$ have been fully resuspended before use. Resuspend by brief vortexing or careful pipetting.

→ Check that your Lysis/Binding Buffer has not precipitated. If any precipitation is observed, warm to room temperature and shake to full resuspension.

→ A rapid lysis in Lysis/Binding Buffer is critical for obtaining un-degraded mRNA. Thawing of frozen material prior to lysis must be avoided.

→ When working with cells isolated with cell-specific Dynabeads®, make sure all the cell-specific Dynabeads® are removed from the lysate before adding Dynabeads® Oligo $(dT)_{25}$

→ Prepare your reverse transcription reaction mix prior to mRNA isolation and keep on ice.

→ We recommend to immediately use the Dynabeads®-mRNA complex for reverse transcription and PCR.

→ Use sterile, RNase-free microtubes and pipette tips.

→ Wear disposable gloves and change them frequently.

→ Refer to the package insert for detailed protocols.

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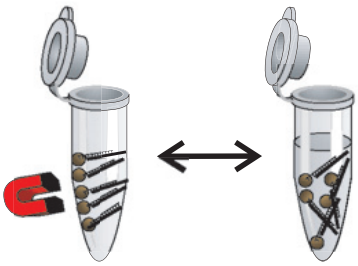
invitrogen bead separations

Add 100 µl Lysis/Binding Buffer to your starting sample and homogenize (e.g. by pipetting) until complete lysis is obtained.

Transfer the lysate to a tube containing 20 µl pre-washed Dynabeads® Oligo $(dT)_{25}$

Mix 5 minutes on a roller at room-temperature for mRNA to hybridize to the oligo-dT on the beads.

Place on magnet and remove supernatant. Add 100 µl Washing Buffer A and resuspend by careful pipetting.



Wash once in 100 µl Washing Buffer A and twice in 100 µl Washing Buffer B. The magnet allows for easy washing and change of buffers.

Resuspend the Dynabeads®-mRNA complex in 100 µl ice-cold Tris-HCl and store on ice for reverse transcription and downstream applications. (Elution is optional at this step.)

Immediately prior to adding the reverse transcription PCR-mix to the Dynabeads® -mRNA complex, apply the magnet and remove the supernatant.

The Dynabeads®-mRNA complex is now ready for reverse transcription and PCR.