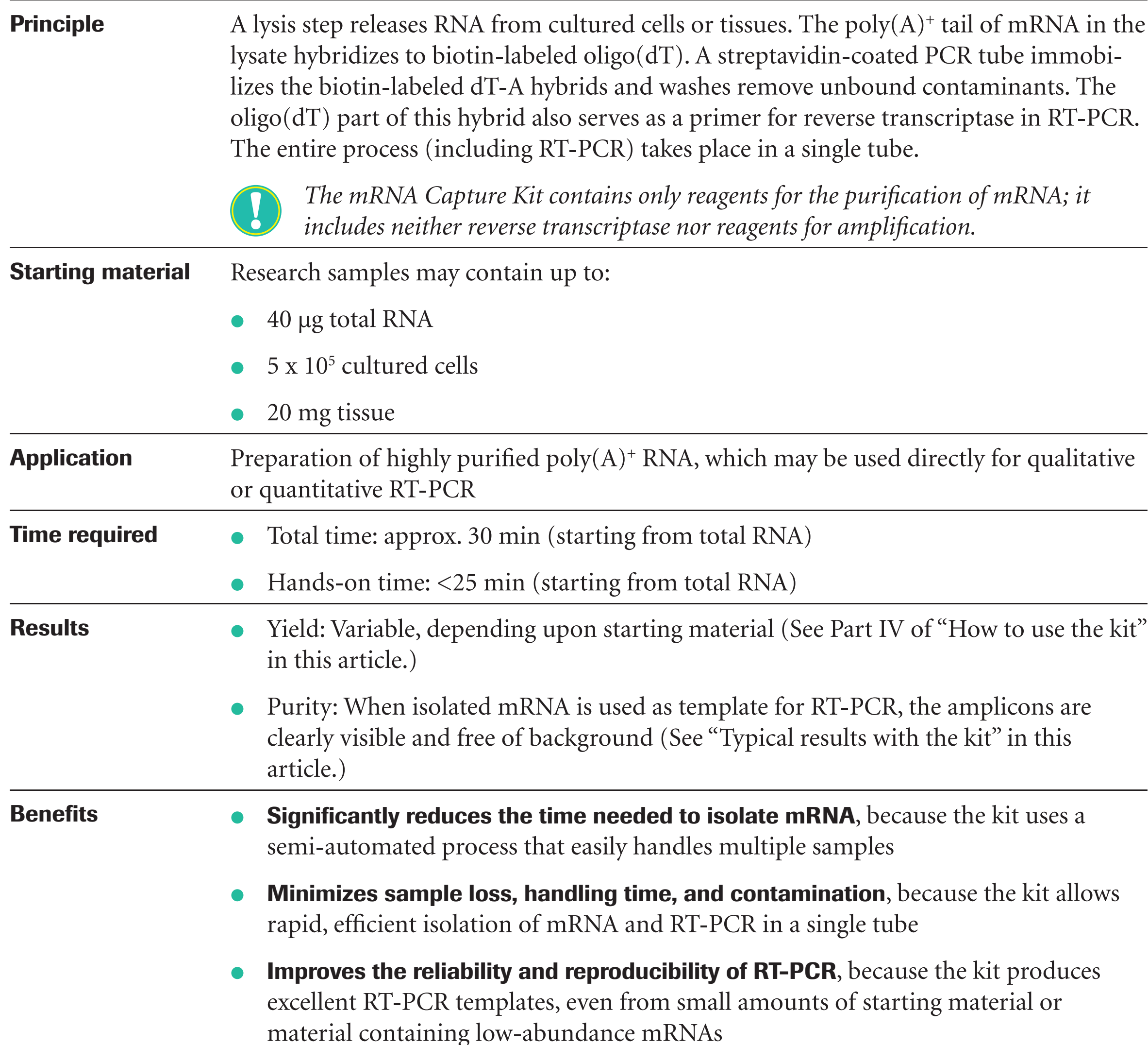
mRNA Capture Kit

for capturing 192 poly(A)$^+$ RNA preparations in PCR tubes

Cat. No. 11 787 896 001



Principle

A lysis step releases RNA from cultured cells or tissues. The poly(A)$^+$ tail of mRNA in the lysate hybridizes to biotin-labeled oligo(dT). A streptavidin-coated PCR tube immobilizes the biotin-labeled dT-A hybrids and washes remove unbound contaminants. The oligo(dT) part of this hybrid also serves as a primer for reverse transcriptase in RT-PCR. The entire process (including RT-PCR) takes place in a single tube.

The mRNA Capture Kit contains only reagents for the purification of mRNA; it includes neither reverse transcriptase nor reagents for amplification.

Starting material

Research samples may contain up to:

- 40 µg total RNA
- 5 x 10^5 cultured cells
- 20 mg tissue

Application

Preparation of highly purified poly(A)$^+$ RNA, which may be used directly for qualitative or quantitative RT-PCR

Time required

- Total time: approx. 30 min (starting from total RNA)
- Hands-on time: <25 min (starting from total RNA)

Results

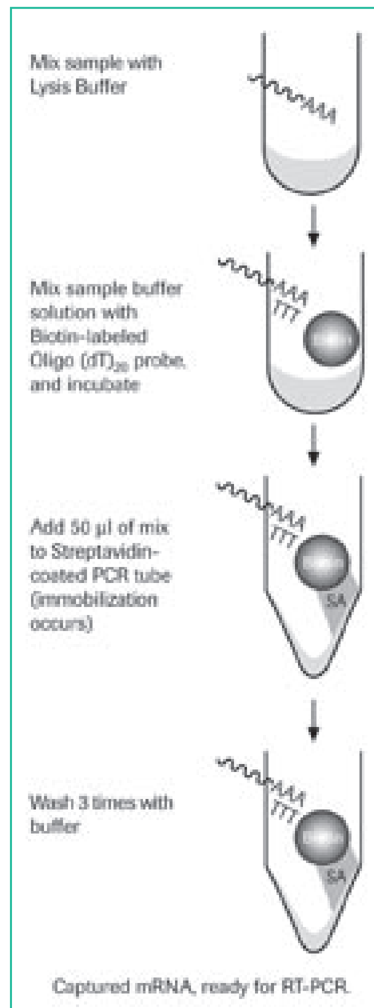
- Yield: Variable, depending upon starting material (See Part IV of "How to use the kit" in this article.)
- Purity: When isolated mRNA is used as template for RT-PCR, the amplicons are clearly visible and free of background (See "Typical results with the kit" in this article.)

Benefits

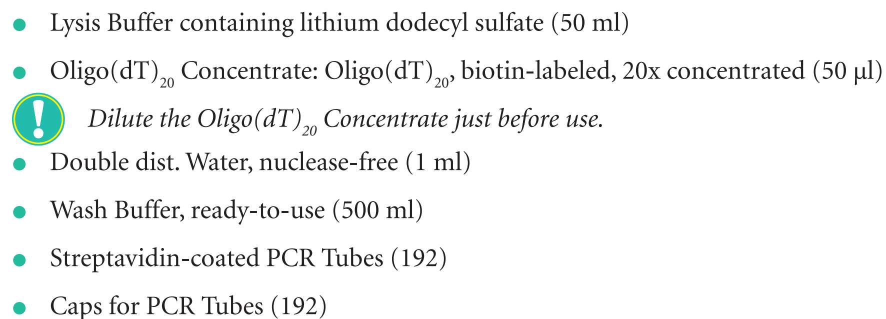
- **Significantly reduces the time needed to isolate mRNA**, because the kit uses a semi-automated process that easily handles multiple samples
- **Minimizes sample loss, handling time, and contamination**, because the kit allows rapid, efficient isolation of mRNA and RT-PCR in a single tube
- **Improves the reliability and reproducibility of RT-PCR**, because the kit produces excellent RT-PCR templates, even from small amounts of starting material or material containing low-abundance mRNAs

How to use the kit

I. Flow diagram



II. Kit contents



- Lysis Buffer containing lithium dodecyl sulfate (50 ml)
- Oligo$(dT)_{20}$ Concentrate: Oligo$(dT)_{20}$, biotin-labeled, 20x concentrated (50 µl)

Dilute the Oligo$(dT)_{20}$ Concentrate just before use.

- Double dist. Water, nuclease-free (1 ml)
- Wash Buffer, ready-to-use (500 ml)
- Streptavidin-coated PCR Tubes (192)
- Caps for PCR Tubes (192)

III. Additional materials needed

- Sterile cups (for preparing dilutions)
- Pipettes with sterile, aerosol-resistant pipette tips
- Syringe fitted with a 21-gauge needle (for tissue and cultured cells only)
- PBS (for preparing cultured cells only)
- Mortar, pestle, liquid nitrogen (for preparing tissue samples only)
- PCR thermal cycler
- Reagents for RT-PCR
- Electrophoresis equipment or other equipment for analyzing PCR products

IV. Typical mRNA content of different samples

Starting material	Total RNA (µg)	mRNA (µg)
Cultured cells (10^7 cells)	30 – 500	0.3 – 25
Tissue (100 mg):		
Mouse brain	200	7
Mouse liver	700	14
Mouse lung	130	10

V. Protocol for capturing mRNA from total RNA, cultured cells, or tissue

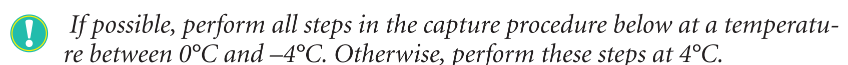
General notes:

- Make sure that all material which contacts the RNA is free of contaminating RNases. For decontamination of equipment, see Appendix (page 219).
- For tips on preventing RNase contamination in this procedure, as well as guidelines for the subsequent RT-PCR procedure, see the package insert provided with the mRNA Capture Kit.

1. To prepare the $Oligo(dT)_{20}$ Working Solution used in Step 3 below, dilute the $Oligo(dT)_{20}$ Concentrate 1:20 with nuclease-free double dist. water.

Prepare the $Oligo(dT)_{20}$ Working Solution just before use; prepare only as much as you need. (Each capture requires 4 µl of Working Solution.)

2. Prepare the sample:
   - Total RNA: Dilute ≤40 µg of total RNA in 200 µl of Lysis Buffer.
   - Cultured cells: Wash cells (≤5 x 10^5) twice with ice-cold phosphate-buffered saline (PBS). Add 200 µl of Lysis Buffer to the cell pellet. Shear DNA mechanically by passing the sample 6 x through a 21-gauge needle.
   - Tissue: Snap freeze ≤20 mg of tissue. Grind frozen tissue to a homogeneous powder in a pre-cooled mortar. Chill 200 µl of Lysis Buffer to ≤0°C in a sodium chloride-ice water bath, then add the frozen powder to the chilled Lysis Buffer. Homogenize the powder suspension by passing it 4 x through a 21-gauge needle. Centrifuge the suspension at 11,000 x *g* for 30 s. Use only the supernatant for the capture procedure.


If possible, perform all steps in the capture procedure below at a temperature between 0°C and –4°C. Otherwise, perform these steps at 4°C.

3. Hybridize the mRNA to biotin-labeled oligo(dT)$_{20}$:
   - Add 4 µl Oligo(dT)$_{20}$ Working Solution to the sample to form the hybridization mix.
   - Incubate the hybridization mix for:
     - EITHER 5 min at 2 to 8°C (if you started with tissue)
     - OR 5 min at 37°C (if you started with cultured cells or total RNA)
4. Immobilize mRNA in the Streptavidin-coated PCR Tubes:
   - Add 50 µl of the hybridization mix to a Streptavidin-coated PCR tube.
     - ! *Use 4 tubes to process entire hybridization mix.*
   - Incubate the hybridization mix for:
     - EITHER 3 min at 2 to 8°C (if you started with tissue)
     - OR 3 min at 37°C (if you started with cultured cells or total RNA)
5. Wash the immobilized sample 3 times. For each wash:
   - Add 250 µl Wash Buffer to the tube.
     - ! *To avoid losing mRNA, do not wash the sample too vigorously.*
   - Remove all Wash Buffer and discard.
     - ! *After the third wash, the captured mRNA in the Streptavidin-coated PCR Tube is ready for RT-PCR.*

VI. Troubleshooting the mRNA Capture protocol

If you get...	Then, the cause may be...	And you should...
No PCR product (after RT-PCR)	Insufficient homogenization of sample material	▶ Eliminate all visible clumps in lysate (Step 1) by homogenization, etc. ! *mRNA trapped inside sample clumps can not be isolated by this procedure.*
	Too much starting material per volume of Lysis Buffer (lysate viscosity too high)	▶ Do not use more than the recommended amount of starting material. ▶ Dilute the lysate (Step 1) before continuing the procedure. ! *The viscosity of the lysate is critical to successful mRNA isolation.*
	Degradation of RNA by contaminating RNase from buffers or equipment	▶ Check all buffers for RNase contamination. ▶ Follow procedures in the package insert for RNase decontamination.
	Degradation of RNA because RNases in sample material were not sufficiently inactivated	▶ Follow the kit procedure exactly as written and be especially careful to: ▶ Work rapidly ▶ Perform indicated steps at ice water temperatures (0 to –4°C)

Typical result with the kit

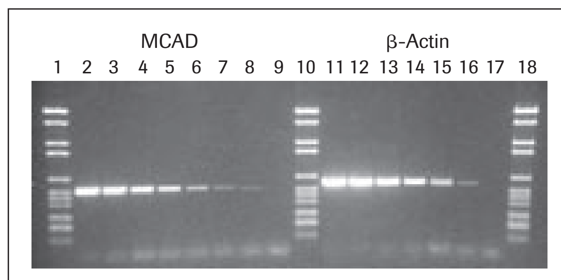


Figure 48: Detection of MCAD and β-actin transcripts in K562 cells. Lysates from 10^6 cells were serially diluted. Messenger RNA from the dilutions was captured in Streptavidin-coated PCR tubes according to the above protocol. RT-PCR was performed in the same PCR tubes with reagents from the Titan One Tube PCR System and primers derived from the human MCAD (Medium-chain acyl-CoA dehydrogenase) and β-actin genes. Amplicons were run on a 1% agarose gel. For details of the procedure and the primers used, see Zoelch and Frey (1996).

Lanes 1, 10, 18: DNA Molecular Weight Marker VI
The remaining lanes contained mRNA from
Lane 2: 1 x 10^4 cells
Lanes 3, 11: 2 x 10^3 cells
Lanes 4, 12: 400 cells
Lanes 5, 13: 80 cells
Lanes 6, 14: 16 cells
Lanes 7, 15: 3.2 cells
Lanes 8, 16: 0.64 cells
Lanes 9, 17: Control (no RT performed)

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Result: The mRNA Capture Kit and Titan One Tube RT-PCR System allow the efficient detection of β-actin and MCAD template RNA's from as few as 3.2 K562 cells.

References

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