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Product Information

GenElute™ mRNA Miniprep Kit

Catalog Numbers **MRN 10, MRN 70**

TECHNICAL BULLETIN

Product Description

The GenElute mRNA Miniprep Kit provides a simple and convenient way to purify polyadenylated mRNA from previously isolated total RNA. Oligo(dT) polystyrene beads bind the poly(A)$^+$ mRNA during a 10 minute incubation. After washing in a microspin filter to remove contaminants, the poly(A)$^+$ mRNA is eluted in 100 µl of buffer. Purification of mRNA from total RNA can be performed in less than 40 minutes. The purified mRNA is ready for Northern analysis, reverse transcription and PCR†, labeling for arrays, and other common applications. Note that if all traces of DNA contamination must be eliminated for RT-PCR, further treatment with Amplification Grade DNase I is recommended, Catalog Number AMPD1.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

RNases are ubiquitous and very stable proteins, which are concerns for any researcher attempting to isolate RNA. Conditions during the binding steps inhibit RNase activity. **Care must be taken not to introduce RNase, especially during the wash and elution steps.** Use RNase-free pipette tips, preferably those having an aerosol barrier. Wear latex or vinyl gloves and change them frequently. Keep bottles and tubes closed when not adding or removing their contents. The references given at the end of this bulletin are good sources of additional information for working with RNA.

Reagents Provided	Catalog Number	MRN 10 10 Preps	MRN 70 70 Preps
Water, Molecular Biology Reagent	W4502	3 ml	20 ml
2X Binding Solution	B1305	3 ml	20 ml
Oligo(dT) Polystyrene Beads	O8512	0.2 ml	1.2 ml
Wash Solution	W2015	15 ml	80 ml
Elution Solution (10 mM Tris-HCl, pH 7.4)	E8151	1.5 ml	8 ml
Spin Filters with Tubes	C9596	10 each	70 each
Dolphin Collection Tubes	D7688	10 each	70 each

Storage

Store the kit at room temperature. If any reagent forms a precipitate, see Preparation Instructions that follow.

Reagents and Equipment Required but Not Provided

- RNase-free 1.5 ml microcentrifuge tubes
- Heating block set at 70 °C
- Microcentrifuge capable of 14,000-16,000 x *g*

Preparation Instructions
Before beginning the procedure, complete the following:

1. Thoroughly mix reagents. Examine for precipitation. If any reagent forms a precipitate, warm at 55-65 °C until the precipitate dissolves and allow to cool to room temperature before use.

2. Transfer approximately 120 µl of Elution Solution per preparation into a microcentrifuge tube and heat to 70 °C in a heating block.

3. Ensure that the oligo(dT) beads are at room temperature; thoroughly vortex prior to use.

4. Determine the quantity and quality of the previously isolated total RNA (starting material).

    a. Read the absorbance at 260 and 280 nm. An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. The concentration of RNA in µg/ml is calculated as follows: A_{260} x dilution factor x 40. A ratio of A_{260}/A_{280} from 1.8 to 2.2 is recommended.

    b. Check the quality of the total RNA starting material by agarose gel electrophoresis. The 28S and 18S rRNA should appear as discrete bands at approximately 5.3 and 2.0 kb, respectively. The mRNA should appear as a light smear, primarily between the 28S and 18S rRNA bands.

5. Use from 5 to 500 µg of total RNA. The volume of the RNA sample should not exceed 250 µl for 15 µl of oligo(dT) polystyrene beads.

Procedure
All steps are carried out at room temperature except as noted. All centrifugation (spin) steps should be performed at maximum speed (14,000-16,000 x g).

1. **Prepare RNA for binding.** Pipette up to 500 µg of total RNA into an RNase-free 1.5 ml microcentrifuge tube (not provided). Adjust the volume to 250 µl with the RNase-free water provided. Add 250 µl of 2X Binding Solution to the total RNA solution and vortex briefly to mix.

2. **Add oligo(dT) beads and denature RNA.** Add 15 µl of oligo(dT) beads and mix the contents by thoroughly vortexing. Incubate the mixture at 70 °C for 3 minutes in a heating block to denature the RNA.

3. **Bind mRNA and collect bead:mRNA complex.** Remove the sample from the heating block and allow to stand at room temperature for 10 minutes. Pellet the oligo(dT) polystyrene beads:mRNA complex by spinning the sample for 2 minutes at maximum speed. Carefully remove and discard the supernatant, leaving behind approximately 50 µl to avoid disturbing the pellet.

4. **First wash.** Resuspend the pellet in 500 µl of Wash Solution by vortexing or pipetting. Pipet the suspension into a GenElute spin filter/collection tube assembly. Ensure that the entire suspension is expelled from the pipette tip and that the pellet is completely removed from tube. Spin for 1- 2 minutes at maximum speed. Remove the column containing beads:mRNA complex from collection tube and discard the flow-through. Return the column into collection tube.

5. **Second wash.** Pipette an additional 500 µl of Wash Solution onto the spin filter. Spin for 1-2 minutes at maximum speed.

6. **First elution.** Transfer the spin filter into a fresh collection tube. Discard the flow-through and the original collection tube. Pipette 50 µl of Elution Solution heated to 70 °C into the center of the spin filter, ensuring that the solution makes contact with the bead:mRNA complex. Incubate for 2-5 minutes at 70 °C. Spin for 1 minute at maximum speed.

7. **Second elution.** Pipette an additional 50 µl of Elution Solution heated to 70 °C into the center of the spin filter, ensuring that the solution makes contact with the bead:mRNA complex. Incubate for 2-5 minutes at 70 °C. Spin for 1 minute. The poly(A)$^+$ mRNA is now in the flow-through eluate and is ready for immediate use or storage at –70 °C.

Results

Determining Yield

Due to the low amount of mRNA (only 1-5% of the total RNA), estimating the concentration and quality of mRNA from mini-preparations spectrophotometrically by measuring absorbance at 260 and 280 nm is not recommended. Typically, the absorbance must be read using the neat sample or with little dilution. As a consequence, most or all of the mRNA isolated will be used. The sample may be recovered for downstream applications, if RNase-free cuvettes are used. However, fluorometric determination with RiboGreen® RNA Quantitation Reagent from Molecular Probes is a more sensitive and practical method, and is recommended.

Expected Yield

The yield of mRNA varies according to the organism and developmental stage. Better results are generally obtained when starting with good quality total RNA, 1-5% of which is mRNA. A typical yield from 100 µg of total RNA from HEK 293 cells is 2-5 µg of mRNA in the 100 µl of Elution Solution.

Ethanol precipitation

Some applications will require a more concentrated mRNA preparation than that prepared with this kit. The mRNA may be concentrated by adding 20 µg glycogen (Product Code G 1767), 0.1 volume of 3 M sodium acetate buffer, pH 5.2 (Product Code S 7899), and 3 volumes of ice cold absolute ethanol (Product Code E 7023), and precipitating overnight at –20 ºC. The mRNA can be stored indefinitely as an ethanol precipitate. Before use, spin the precipitated mRNA at 14,000-16,000 x *g* at 2-8 ºC for 15 minutes. Carefully remove the supernatant, wash the pellet with 70% ethanol, and spin at maximum speed for 3-5 minutes. Carefully remove the supernatant and allow the tube to air dry while lying flat with the lid open for 30 minutes. Once dry, the pellet can be dissolved in RNase-free molecular biology reagent water (Product Code W 4502).

Agarose gel electrophoresis

The mRNA may be fractionated on a denaturing formaldehyde agarose gel as described by Farrell.[1] Staining with SYBR® Green II, Catalog No. S9305, is recommended, because formaldehyde gels stained with SYBR Green do not require destaining. The mRNA should appear as a series of very closely spaced bands or a smear at 0.5 kb or greater. More intense bands at approximately 5.3 and 2.0 kb are residual 18S and 28S rRNA.

Northern analysis

Integrity of the mRNA can be analyzed by performing a Northern blot of the formaldehyde denaturing agarose gel followed by hybridization with a probe specific for an mRNA present in the population. See Related Products list for products and procedures to perform Northern blots, label probes and perform hybridization. Intact mRNA should result in a discrete band or bands, with no smearing.

RT-PCR

While most DNA is eliminated during mRNA isolation, no single procedure removes 100% of the DNA. Because PCR can detect even a single molecule of DNA, RNA samples should be digested with amplification grade DNase I (Product Code AMP-D1) before RT-PCR and parallel samples should be assayed without adding reverse transcriptase. These precautions are especially recommended if PCR primers do not span an intron, or if pseudogenes that lack the intron may be present in the target cells or tissue.

References

1. Farrell, Robert E., Jr. *RNA Methodologies*, 2nd Edition, pp. 37-53 and 153-156 (Academic Press, NY, 1998) (Product Code Z35,035-4)
2. Ausubel, F. M., *et al*., *Current Protocols in Molecular Biology*, Sections 4.1-4.10 (John Wiley & Sons, NY, 1995)
3. Sambrook, J., *et al*., *Molecular Cloning: A Laboratory Manual*, 2nd ed., pp. 7.3-7.8 (Cold Spring Harbor Laboratory Press, Plainview, NY 1989)

Troubleshooting Guide

Problem	Cause	Solution
Low yield of mRNA	RNA was derived from cells or tissues that had low mRNA levels	Yields will vary greatly among different cell and tissue types. See "Expected Yield" in the Results section.
	Starting material is not pure	Ensure the the starting total RNA does not contain any components (such as RNase) that would inhibit the proper isolation of poly(A)$^+$ mRNA during this procedure. We recommend using RNA isolated with GenElute Mammalian Total RNA Kits (Product Codes RTN 10, RTN 70 and RTN 350).
	Too much or too little starting material	Use 5 to 500 µg of total RNA for 15 µl of oligo(dT) polystyrene beads.
	Elution Solution was not pre-heated or samples were not incubated at 70 ºC	Transfer approximately 120 µl of Elution Solution per preparation into a microcentrifuge tube and heat to 70 °C in a heating block before starting the procedure. Incubate bead:mRNA complex with Elution Solution for 2-5 minutes at 70 °C before spinning.
	RNA was degraded in starting material	Check the integrity of total RNA by agarose gel electrophoresis before isolating mRNA.
Degraded mRNA	RNase was introduced during the procedure.	Pay special attention to precautions for handling RNA samples and related lab equipment listed at the beginning of this bulletin and in references 1-3.
	Starting total RNA is contaminated	Be certain the procedure used to isolate the total RNA removes any potential contaminants.
Excessive rRNA contamination	Abundance of rRNA is high; sequence of rRNA contains poly(A) regions	Detectable amounts of rRNA are expected. Non-specific binding to oligo(dT) will occur due to the vast excess of rRNA over mRNA. Also, poly(A) regions in rRNA can bind specifically. If more highly enriched mRNA is desired, repeat the purification as follows: Adjust the volume of eluted mRNA to 250 µl with RNase-free water. Add 250 µl of 2X Binding Solution and continue with the procedure starting at step 2.
	Miniprep capacity was exceeded	Re-purify as above. For future preparations, use a smaller quantity of RNA starting material.
Poor results in downstream applications	Improper storage or handling of eluate	Store eluted mRNA in Elution Solution at -70 ºC or as an ethanol precipitate at –70 ºC until needed. When mRNA solution is out of the –70 °C environment, it should be placed on ice until needed.

Related Products	Catalog Number	Related Products	Catalog Number
GenElute Mammalian Total RNA Kits	RTN10 RTN70 RTN350	Deoxyribonuclease I, Amplification Grade	AMPD1
GenElute Direct mRNA Miniprep Kits	DMN10 DMN70	Enhanced Avian RT PCR Kits	RTPCR20 RTPCR100
Ethanol, Absolute, Molecular Biology Grade	E7023	Ethidium Bromide, Aqueous Solution, 10 mg/ml	E1510
Glycogen, Molecular Biology Grade	G1767	SYBR Green II, 10,000X concentrate	S9305
3 M Sodium Acetate Buffer, pH 5.2, Molecular Biology Grade	S7899	Formaldehyde, Molecular Biology Grade	F8775
RNA Sample Loading Buffer	R1386 R4268	MOPS-EDTA-Sodium Acetate Buffer	M5755
Agarose, Molecular Biology Grade	A9539	RNA markers, 0.2-10 kb	R7020
Enhanced Avian Reverse Transcriptase	A4464	RNA/cDNA Inspector Kit	INSP1
Deoxynucleotide (dNTP) Mix	D7295	PerfectHyb™ Plus Hybridization Buffer	H7033
Taq DNA Polymerase	D1806	Uniscript™ T7 Transcription kit	UST7
JumpStart™ Taq DNA Polymerase	D9307	CDP-Star® Universal Detection kit	UALK

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