GE Healthcare

illustra™ QuickPrep *Micro* mRNA Purification Kit

Product booklet

Code: 27-9255-01 (24 preps)

Page finder

1. Legal

Product use restriction

The **QuickPrep™ *Micro* mRNA Purification Kit** components have been designed, developed, and sold for **research purposes only.** They are suitable for ***in vitro* uses only.** No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the **QuickPrep *Micro* mRNA Purification Kit** for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Protocol requires the use of Guanidinium Thiocyanate, Cesium Trifluoroacetate, and potentially Diethyl Pyrocarbonate. Wear gloves and safety glasses.

We recommend that this product and components are handled only by those persons who have been trained in laboratory techniques and that they are used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

All kit components should be stored at 2–8°C.

2.3. Expiry

For expiry date, please refer to outer packaging label.

3. Components

3.1. Kit contents

Table 3.1. QuickPrep *Micro* mRNA Purification Kit contents

Pack Size: 24 Preps Cat. No.: 27-9255-01	Volume	Quantity
Oligo(dT)-Cellulose	25 ml	Sufficient for 24 preps
Extraction Buffer	20 ml	1 vial
High Salt Buffer	125 ml	1 vial
Low Salt Buffer	100 ml	1 vial
Elution Buffer	50 ml	1 vial
Glycogen Solution	300 µl	1 vial
Potassium Acetate Solution	1.25 ml	1 vial
MicroSpin Columns	-	25

3.2. Reagents and materials to be supplied by user

All plastic or glass labware that may come into contact with the sample should be RNase-free (5).

- Mechanical or manual tissue homogenizer (optional)
- Centrifuge with a swinging-bucket rotor; this must be capable of accommodating a 15 ml centrifuge tube with a spin column inserted into it.
- TE Buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA)
- 95–100% ethanol
- RNase-free water or DEPC-treated water

4. Description

4.1. The basic principle

QuickPrep *Micro* mRNA Purification Kit is designed for the direct isolation of polyadenylated RNA from small amounts of eukaryotic cells or tissues, bypassing the need for intermediate purification of total RNA.

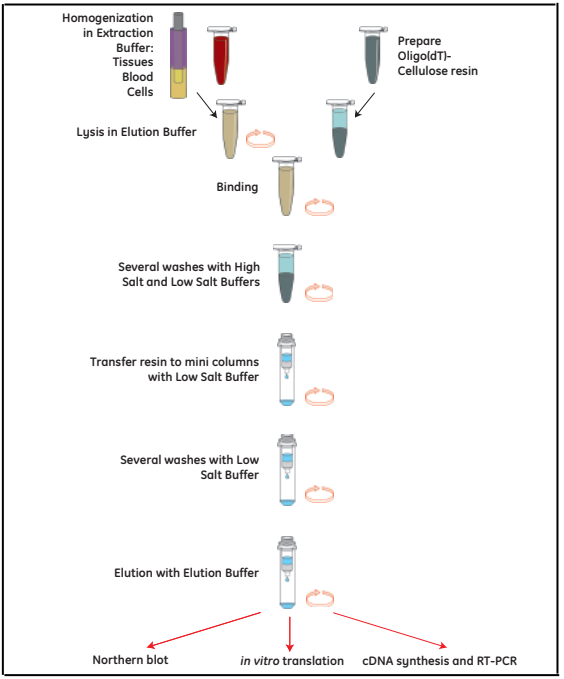


Figure 4.1. Overview of the **QuickPrep *Micro* mRNA Purification Kit** procedure

QuickPrep *Micro* mRNA Purification Kit combines the disruptive and protective properties of guanidine thiocyanate (GTC) with the speed and selectivity of Oligo(dT)-Cellulose chromatography in a spin column format (1, 2, 3). The kit is designed for the rapid isolation of mRNA direct from small amounts of eukaryotic cells or tissue without the need for intermediate purification of total RNA. Starting with cultured cells, mRNA can be purified in just 15 minutes; purification of mRNA from tissue samples will take several minutes longer, due to the need for homogenization of the tissue. The kit can be used to isolate mRNA from as few as one cell or as many as 1×10^7 cells (~ 100 mg of tissue).

The protocol for mRNA purification using a microcentrifuge is given in Section 7 and is outlined in Figure 4.1. The following summarizes the steps involved when using this protocol. Briefly, the tissue or cells are extracted in a buffered solution containing a high concentration of GTC, ensuring the rapid inactivation of endogenous RNases. The extract is then diluted with Elution Buffer, reducing the GTC concentration to a carefully selected level - low enough to allow efficient hydrogen bonding between poly(A) tracts on the mRNA molecules and Oligo(dT) attached to cellulose, but high enough to maintain complete inhibition of RNases. The dilution causes a number of proteins to precipitate, giving an initial purification.

The extract is then clarified by a short centrifugation step. The supernatant is transferred to a microcentrifuge tube containing Oligo(dT)-Cellulose. After incubating for several minutes to allow the poly(A) RNA to bind to the Oligo(dT)-Cellulose, the tube is centrifuged and the supernatant is removed. The pelleted material is then washed sequentially with High Salt Buffer and Low Salt Buffer, each wash being accomplished by a process of resuspension and brief re-centrifugation.

After the last wash with Low Salt Buffer, the pelleted Oligo(dT)-Cellulose is brought up in a small volume of Low Salt Buffer. The slurry is transferred to a MicroSpin™ Column placed within a microcentrifuge tube, and the column is washed with Low Salt Buffer. Alternatively, the streamlined washing steps in Section 7.3. may be used, which will yield slightly less pure mRNA, but shortens the isolation time by eliminating some of the washing steps. Finally, the polyadenylated material is eluted with one or two applications of prewarmed Elution Buffer.

4.2. Kit specifications

The starting point for this procedure is total RNA extracted from eukaryotic cells or tissue. Each purification will yield a maximum of 6 µg of mRNA. Purity will vary depending on the sample, with at least 50% and typically 90% or more of the isolated RNA being polyadenylated. The mRNA isolated with the kit is essentially free of DNA and protein contamination.

Table 4.1. QuickPrep *Micro* mRNA Purification Kit Technical Specifications at a Glance

	Tissue or Cells
Sample size	< 100 mg of tissue or ≤ 10^7 cells
Elution volume	200 µl
mRNA yield	up to 6 µg
Purity	≥ 90% will be polyadenylated RNA
Time/Prep	15 min/prep

mRNA purified with the kit has been used directly in first-strand cDNA synthesis prior to PCR without dilution or concentration. Note, however, that the maximum concentration of mRNA likely to be obtained is 15 µg/ml, which may be insufficient for some downstream applications. For Northern analysis, standard cDNA synthesis and *in vitro* translation, we recommend that the RNA be concentrated prior to use. The kit contains both potassium acetate and glycogen for precipitation of the mRNA when required (see Section 7.2.).

5. Preparation of working solutions

Extraction Buffer and Potassium Acetate solutions are irritants and should be handled with care.

The reagents are packaged as “stock solutions”. We recommend preparing aliquots of each reagent in RNase-free tubes before beginning the isolation procedure. This will help avoid possible contamination of the stock reagents.

5.1. 0.1% DEPC treated water

Prepare a 0.1% (v/v) solution of **Diethyl Pyrocarbonate (DEPC)** in distilled water, shake vigorously, and allow to stand overnight at room temperature. Autoclave the solution on the following day with the cap loosened. Commercially available RNase-free water may be used instead of DEPC-treated water in this protocol.

5.2. TE buffer

10 mM **Tris-HCl, pH 7.4,** 1 mM **EDTA**, prepared with DEPC-treated or RNase-free water. Autoclave the buffer after the addition of the **Tris**. Commercially available **TE (pH 7.4)** with this formulation may also be used.

5.3. 5.5 M Guanidinium thiocyanate

The 5.5 M **Guanidinium Thiocyanate** solution can be prepared by combining 65 g of **guanidinium thiocyanate** with 2.5 ml of 1 M **sodium citrate, pH 7.0,** and 0.5 g of **sodium lauryl sarcosine** in distilled water to a final volume of 100 ml (while warming and stirring). The final pH should be adjusted to 7.0 with a small amount of **1 N Sodium Hydroxide (NaOH)**. At this point the solution can be stored at 2–8°C for several days.

Immediately prior to use, heat the solution to 37°C, cool to room temperature and add **β-mercaptoethanol** to a final concentration of 0.2 M. Clarify the solution by filtration through a 0.2 µm filter.

5.4. Preparation of Oligo(dT)-Cellulose

Before using a column, snap off the bottom closure. This portion can then be inverted to plug the column.
Gently swirl the **Oligo(dT)-Cellulose** slurry to obtain a uniform suspension. Aliquot 1 ml of **Oligo(dT)–Cellulose** into individual microcentrifuge tubes for each purification to be performed.
Centrifuge tubes in step 1 of Section 7, (see page 16, Cell Lysis).

6. Handling, preparation, and storage of starting materials

Approximately 20–30 minutes before sample extraction, remove the Extraction Buffer from 4°C storage and place at 37°C. Shake the bottle occasionally until all the crystalline material is dissolved. If the crystalline material persists, place the bottle at 55°C and shake occasionally. If it is difficult to get the final crystals into solution, simply allow the crystalline material to settle and pipette the solution away from the crystals. This will not result in any deleterious effects on buffer performance. Cool Extraction Buffer to room temperature before use.

During extraction, disrupted cells may appear to clump together, or the homogenate may become quite viscous. Neither of these phenomena will affect the outcome of the extraction. Continue the procedure uninterrupted.

Use a microcentrifuge that can generate a centrifugal force between 5 000 × *g* and 16 000 × *g*. The method was developed using an Eppendorf Model 5415, where at full speed, it provides a g-force of 16 000.

Standard 1.5 ml and 2.0 ml microcentrifuge tubes were used in the development of the **QuickPrep *Micro* mRNA Purification Kit**. It is convenient to use microcentrifuge tubes with attached caps for the initial binding and washing procedures. A 10-second pulse at 16 000 × *g* is sufficient to pellet the Oligo(dT)-Cellulose.

Avoid ribonuclease contamination, especially those present on the skin, by wearing clean gloves at all times and by autoclaving or DEPC-treating all glass and plasticware. Also, prepare buffers with DEPC-treated or RNase-free water, and autoclave.

Tissues (up to 0.1 g)

Place the tissue in a chilled homogenizer (either manual or mechanical) and add **0.4 ml of Extraction Buffer.** Homogenize the tissue until it is a uniform suspension. The tissue should be well homogenized in a 7 ml homogenizer using 10–20 strokes with a smaller pestle followed by 10 strokes with a larger pestle. Avoid the generation of excess heat or foam.

Cultured cells (up to 10^7 cells)

Free adherent cells from anchorage by standard methodology (4,5) and suspend them in a small volume of isotonic buffer. Count the cells using a hemocytometer (5).

Place an aliquot of cell suspension containing up to 1×10^7 cells in a microcentrifuge tube and pellet by centrifugation. Remove the supernatant. Add **0.4 ml of Extraction Buffer** to the pelleted cells. Vortex until a homogenous solution is achieved.

Blood samples (~ 25 µl)

Blood samples can be used directly; there is no need to pellet them prior to extraction.

7. Protocols

Preparation of the Oligo(dT)-Cellulose columns is described in Section 5.4.

For tissue that is difficult to homogenize, we recommend adding **0.6 ml of Extraction Buffer** (rather than 0.4 ml)**.** With this larger volume 2 ml microcentrifuge tubes are required for both the extraction and the binding steps. In the text below, the volumes in parentheses should be used if the extraction was performed by adding **0.6 ml of Extraction Buffer**. If only one volume is given, it pertains to all samples, regardless of starting volume.

Resin may be inadvertently removed during buffer aspiration. If the amount is minimal, it should not affect the purification. If a large amount of resin is accidentally removed during washing, the aspirated liquid should be returned to the tube and the centrifugation and buffer removal steps repeated.

Occasionally, a small amount of resin may leak through the frit of the MicroSpin Column. This should not affect the success of the mRNA purification.

The capacity of the QuickPrep system is finite (refer to the Product Description). Overloading the system will result in a decrease in the quality of the mRNA isolated (as measured by the A_{260}/ A_{280} ratio).

7.1. mRNA purification

1. Cell Lysis

Dilute samples from Section 6 with **0.8 ml (or 1.2 ml) of Elution Buffer** and mix thoroughly. Homogenize briefly (or vortex for cultured cells).

+ 0.8 ml (1.2 ml) Elution Buffer

Transfer the homogenate into a sterile microcentrifuge tube.

Mix

Place the remaining Elution Buffer at 65°C until needed in step 8.

Transfer lysate to tube

Centrifuge the lysate and the tube of Oligo(dT)-Cellulose from Section 5 for **1 min at max. speed (5 000 – 16 000 × g) at RT.**

Spin 1 min
max. speed

2. Binding

Aspirate the buffer from the Oligo(dT)-Cellulose pellet. Transfer **1 ml (1.5 ml)** of the **cleared cellular homogenate** to the top of the **Oligo(dT)-Cellulose** pellet.

Close the tube and gently **mix by inversion for 3 min** either manually or by placing it on a rocking table or similar device.

Discard storage buffer

+ 1 ml (1.5 ml) cleared homogenate

Mix by inversion 3 min

The Oligo(dT)-Cellulose may form small clumps, but this will not affect the procedure.

Spin 10 sec max. speed

Centrifuge at **maximum speed (~ 16 000 × *g*) for 10 sec.**

Aspirate supernatant.

3. Washes

The following protocol is designed for isolating mRNA that is essentially free of contaminating proteins, nucleic acids and carbohydrates. If this is not required, use the modified protocol in Section 7.3.

High Salt Wash

Add **1 ml of High Salt Buffer** and resuspend the Oligo(dT)-Cellulose by inversion. Tap the bottom of the tube.
Centrifuge for **10 sec** at **max. speed** and aspirate supernatant.

+ 1 ml High Salt Buffer

Spin 10 sec, max. speed

Discard supernatant

Repeat the wash with **High Salt Buffer** 4 more times, as described above, for a total of 5 washes.

Repeat 4 more times

Low Salt Wash

+ 1 ml of Low Salt Buffer

Apply **1 ml of Low Salt Buffer** to the **Oligo(dT)-Cellulose** pellet and resuspend the resin by inversion. Tap the bottom of the tube.
Centrifuge at **350 × *g* for 2 min**. Aspirate and discard the supernatant.

Spin 2 min
350 × *g*

Discard supernatant

Repeat **once** for a total of 2 washes.

Repeat once more

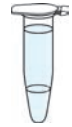
4. Prepare Column

Once the resin is poured into a MicroSpin Column, the centrifugation times should be consistent (5 sec each). This is most easily monitored using a digital timer. Use of 2 ml microcentrifuge tubes decreases the number of times the collection tube must be removed and emptied.

Add **0.3 ml Low Salt Buffer** to the **resin** and mix by pipetting. Transfer the slurry to a MicroSpin Column, placed in a microcentrifuge tube. Centrifuge at **max. speed for 5 sec.** Aspirate and discard the supernatant and place the column back in the collection tube.

Gently add **0.5 ml of Low Salt Buffer.** Avoid disturbing the cellulose bed. Centrifuge at **max. speed for 5 sec.**

Repeat this wash step **twice** more, discarding the eluate between each step, if necessary, for a total of 3 washes.



+ 0.3 ml Low Salt Buffer to resin

Spin 5 sec
max. speed

Discard supernatant

+ 0.5 ml Low Salt Buffer to resin

Spin 5 sec
max. speed

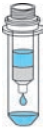
Repeat twice

5. Elution

For the final elution step, we suggest using screw-top microcentrifuge tubes.

Place the column in a sterile screw-top microcentrifuge tube. Add **0.2 ml of prewarmed (65°C) Elution Buffer** to the top of the resin bed. Centrifuge at **max. speed for 5 sec.**

+ 0.2 ml prewarmed Elution Buffer to resin



Spin 5 sec
max. speed

Remove the column and place the tube containing purified mRNA on ice; the sample is now ready for quantitation, precipitation or a downstream application.

The 0.2 ml eluate will contain approximately 80–90% of the recoverable mRNA. The majority of the remaining mRNA can be captured with a second elution step (see below). A second elution step will result in a more dilute mRNA sample which may need to be precipitated in order to concentrate the mRNA prior to use in a downstream application.

Repeat if desired

Optional: Add a second **0.2 ml prewarmed Elution Buffer** to the top of the resin bed and centrifuge as described above.

7.2. Support protocol QuickPrep *Micro* mRNA purification kit: mRNA concentration

1. Prepare sample

Add **40 µl (1/10 volume) of Potassium Acetate Solution** (provided) and **10 µl of Glycogen Solution** (provided) to the 400 µl of eluted mRNA sample. Add **1 ml of chilled 95% ethanol** (2–2.5 volumes) and incubate the sample at -20°C for a minimum of 30 min.

The amount of glycogen should remain constant regardless of volume.

2. Collect Sample

Centrifuge at max. speed in a microcentrifuge at **4°C for 5 min**. If the RNA is not to be used immediately, store it in this precipitated state (in ethanol) at -80°C.

3. Resuspend mRNA Pellet

Decant the supernatant and invert the tube over a clean paper towel. Gently tap the tube on the towel to facilitate the removal of excess liquid. Redissolve the precipitated RNA in an appropriate volume of **Elution Buffer** or **RNase-free water.**

To determine the appropriate resuspension volume, consider the RNA concentration desired, the concentration before precipitation and the volume of the sample subjected to precipitation.

However, the percentage of the RNA recovered after precipitation will depend on the total amount present. With 10 µg of RNA, for example, approximately 70% will be recovered. You may therefore wish to redissolve the pellet in a volume 25–50% smaller than would be required if all of the RNA were recovered.

7.3. Support protocol QuickPrep *Micro* mRNA purification kit: streamlined washing steps

The streamlined protocol can be utilized when downstream applications do not require the highest purity mRNA. The following protocol may be substituted for the washing steps described in Section 7.1 steps 3 through 5.

1. Washes

High Salt Wash

Add **1 ml of High Salt Buffer**. Close the lid of the tube and resuspend the **Oligo(dT)-Cellulose** by inversion. Tap the bottom of the tube. Centrifuge at **max. speed for 10 sec.** Aspirate the supernatant.

Repeat this wash step, as described above, **1–2 more times.**

Purity will vary depending on sample. As a general rule, two High Salt washes will remove approximately 80–90% of contaminating proteins and nucleic acids. A third High Salt wash can be used to achieve even greater purity. Five washes may be needed to completely remove carbohydrates.

Low Salt Wash

Add **1 ml of Low Salt Buffer** to the **Oligo(dT)–Cellulose pellet** and close the lid. Resuspend the resin by inversion. Tap the bottom of the tube. Pellet the resin by centrifugation at **max. speed for 10 sec.** Aspirate the supernatant.

Repeat this wash step **1–2 more times.**

The number of Low Salt washes should equal the number of High Salt washes performed in the previous step.

2. Prepare Column
Resuspend the **resin** in **0.3 ml of Low Salt Buffer** by pipetting and transfer the slurry to a MicroSpin Column placed in a microcentrifuge tube. Centrifuge at **max. speed for 5 sec.**

3. Elution
Place the column in a **sterile screw-top microcentrifuge tube.** Add **0.2 ml of prewarmed Elution Buffer** to the top of the **resin bed.** Centrifuge at **max. speed for 5 sec.**

Remove the column and place the tube containing purified mRNA on ice for quantitation, precipitation or direct use in a downstream application.

The 0.2 ml eluate will contain approximately 80–90% of the recoverable mRNA. The majority of the remaining mRNA can be captured with a second elution step (see below). A second elution step will result in a more dilute mRNA sample which may need to be precipitated in order to concentrate the mRNA prior to use in a downstream application.

Optional: Add a second **0.2 ml aliquot of warm Elution Buffer** to the top of the **resin bed** and centrifuge as described above.

8. Appendix
8.1. Quantitation of mRNA

Procedure for pre-treating quartz cuvettes:
Soak 0.5 ml quartz glass cuvettes in a concentrated HCl:methanol solution (1:1) for 1 hr. Then rinse the cuvettes several times in RNase-free water.

For spectrophotometer quantitation, we recommend transferring the samples to microcentrifuge tubes and spinning at max. speed in a microcentrifuge for 1 minute to remove the residual cellulose resin. Carefully pipet the supernatant into a clean RNase-free microcentrifuge tube. Discard the original tube.

We suggest that you determine the concentration of the mRNA via spectrophotometry using undiluted eluate, placing the sample in a pretreated cuvette and recovering it for a downstream application. A spectrophotometer such as the NanoVue™ will help minimize that amount of mRNA sample necessary for this purpose. The absorbance reading at 260 nm must be between 0.05 and 2.0 to reflect the RNA concentration accurately. If the absorbance at 260 nm is > 2.0, the sample should be diluted to determine the RNA concentration. The minimum absorbance which can be relied upon to be accurate is 0.05, equivalent to an RNA concentration of 2 µg/ml.

Calculate the concentration of the RNA present in the eluate ([RNA]) using the standard formula (5):

$[RNA] = A_{260} \times D \times 40$ µg/ml
D = final dilution factor (in the simplest case above, this would be 1).

If the absorbance of the diluted sample is greater than or equal to 0.5, the sample may be used directly for cDNA synthesis utilizing the cDNA Synthesis Kit (27-9260-01), TimeSaver™ cDNA Synthesis Kit (27-9262-01), First-Strand cDNA Synthesis Kit (27-9261-01), or Ready-To-Go™ T-Primed First-Strand Kit (27-9263-01). If the absorbance is less than 0.5, the sample must be precipitated and redissolved at a higher concentration to ensure efficient cDNA synthesis utilizing any of these kits.

8.2. Composition of kit components

Table 8.1. Composition of Kit Components

Oligo(dT)-Cellulose	Oligo(dT)-Cellulose (25 mg/ml) suspended in a buffer containing 0.15% Kathon™ CG/1CP Biocide
Extraction Buffer	Buffered aqueous solution containing guanidinium thiocyanate and N-lauroyl sarcosine
High Salt Buffer	10 mM TrisHCl (pH 7.5), 1 mM EDTA, 0.5 M NaCl
Low Salt Buffer	10 mM TrisHCl (pH 7.5), 1 mM EDTA, 0.1 M NaCl
Elution Buffer	10 mM TrisHCl (pH 7.5), 1 mM EDTA
Glycogen Solution	5–10 mg/ml glycogen in DEPC-treated water
Potassium Acetate Solution	2.5 M potassium acetate (pH 5.0)
MicroSpin Columns	polypropylene mini columns, each fitted with a frit

8.3. References

1. Chirgwin, J. M. *et al., Biochemistry* **18**, 5294 (1979).

2. Pharmacia P-L Biochemicals, *Analects* **17.4** (1989).

3. Pharmacia P-L Biochemicals, *Analects* **16.2**(1988).

4. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, second edition (1989).

5. Ausubel, F.M. *et al.* (Ed.), *Short protocols in molecular biology: a compendium of current protocols in molecular biology* (5th ed), John Wiley and Sons, Inc. Indianapolis, IN (2002).

8.4. Troubleshooting guide

Problem: Oligo (dT)–Cellulose resin leaking from column

Possible cause	Suggestions
Column design	• A small amount of Oligo (dT)–Cellulose resin will normally leak from the column during the column draining steps. Any resin that may leak will be removed during the draining and washing steps of the column preparation and will not affect the integrity of the mRNA sample.

Problem: Low yield

Possible cause	Suggestions
Sample material	• Tissue samples that contain high amounts of connective tissue, yeast cells and certain plant tissues are known to be difficult to disrupt, and special care must be taken to ensure that the cells are thoroughly disrupted when extracting RNA.

Problem: Samples are not of sufficient purity

Possible cause	Suggestions
Overloaded columns	• Applying more than the maximum amounts of cells or tissue to the columns will result in a loss of purity as reflected by a decrease in the A_{260}/A_{280} ratio. The resin will appear highly viscous, and mucous-like when the sample is mixed in the column.
Sample material	• Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption.

9. Related products

RNAspin Mini RNA Isolation Kit	25-0500-70
RNAspin Midi RNA Isolation Kit	25-0500-73
RNAspin 96 RNA Isolation Kit	25-0500-74
QuickPrep mRNA Purification Kit	27-9254-01
mRNA Purification Kit	27-9258-01
Oligo(dT)-Cellulose Type 7	27-5543-02
Cesium Trifluoroacetate	17-0847-02
TimeSaver cDNA Synthesis Kit	27-9262-01
CodeLink™ Expression Assay Reagent Kit	320012
Ready-To-Go RT-PCR Beads	27-9266-01
RT-PCR Master Mix	E78370
First-Strand cDNA Synthesis Kit	27-9261-01
Ready-To-Go You-Prime First-Strand Beads	27-9264-01
Ready-To-Go T-Primed First-Strand Kit	27-9263-01
dNTP Set	28-4065-51
RNAguard Ribonuclease Inhibitor (Human Placenta)	27-0815-01
AMV Reverse Transcriptase	E70041Y
M-MLV Reverse Transcriptase	E70456Y
Amersham Hybond™-N+	RPN119B
Rediprime™ II DNA labeling system	RPN1633
AlkPhos Direct™ labeling and detection system	RPN3692
Amersham Hyperfilm™ MP	RPN1677K
Amersham Hyperfilm ECL™	RPN2114K

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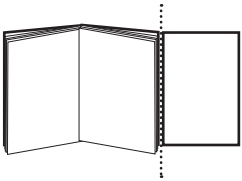

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QuickPrep *Micro* mRNA Purification Kit

Protocol reminder card 27-9255-01

mRNA isolation from small amounts of eukaryotic cultured cells and tissues

1. **Sample homogenization**

Tissues (≤ 0.1 g)

+ 0.4 ml (0.6 ml) Extraction Buffer

Homogenize

Cells (≤ 10^7 cells)

Pellet cells

Remove supernatant

+ 0.4 ml Extraction Buffer

2. **Prepare the Oligo(dT)-Cellulose**

Aliquot 1 ml Oligo(dT)-Cellulose per tube

Spin 1 min max. speed

3. **Cell Lysis**		+ 0.8 ml (1.2 ml) Elution Buffer Homogenize/vortex
		Transfer lysate to clean tube
		Spin 1 min max. speed
4. **Binding**		Discard buffer from Oligo(dT)-Cellulose pellet + 1 ml (1.5 ml) cleared homogenate to resin pellet
		Mix by inversion, 3 min Spin 10 sec max. speed
		Discard supernatant
5. **High Salt Washes**		+ 1 ml High Salt Buffer Resuspend resin
		Spin 10 sec max. speed Discard supernatant **Repeat 4 more times**
6. **Low Salt Washes**		+ 1 ml Low Salt Buffer Resuspend resin
		Spin 2 min 350 × *g* Discard supernatant **Repeat once**

7. **Prepare column**

+ 0.3 ml Low Salt Buffer

Mix by pipetting

Transfer slurry to MicroSpin column

Spin 5 sec max. speed

Discard supernatant

+ 0.5 ml Low Salt Buffer

Spin 5 sec max. speed
Repeat twice

8. **Elution**

Transfer column to sterile screw-top tube

+ 0.2 ml 65°C Elution Buffer

Spin 5 sec max. speed
Repeat if desired

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