GE Healthcare

illustra™ mRNA Purification Kit

Product booklet

Code: 27-9258-01 (2 preps)
27-9258-02 (4 preps)

Page finder

1. Legal

Product use restriction

The **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 **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.

Diethyl Pyrocarbonate. Wear gloves and safety glasses.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the 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. mRNA Purification Kit contents

Pack Size: Cat. No.:	2 preps 27-9258-01	4 preps 27-9258-02
Oligo(dT)-Cellulose columns	4	8
High Salt Buffer	2 vials	4 vials
Sample Buffer	2 vials	4 vials
Low Salt Buffer	2 vials	4 vials
Elution Buffer	2 vials	4 vials
Glycogen Solution	1 vial	1 vial
Protocol	1	1

3.2. Reagents to be supplied by user

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

- RNase-free or DEPC-treated water
- TE Buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA)
- 95-100% ethanol

4. Description

4.1. The basic principle

The **mRNA Purification Kit** is designed for the rapid purification of polyadenylated RNA from eukaryotic total RNA.

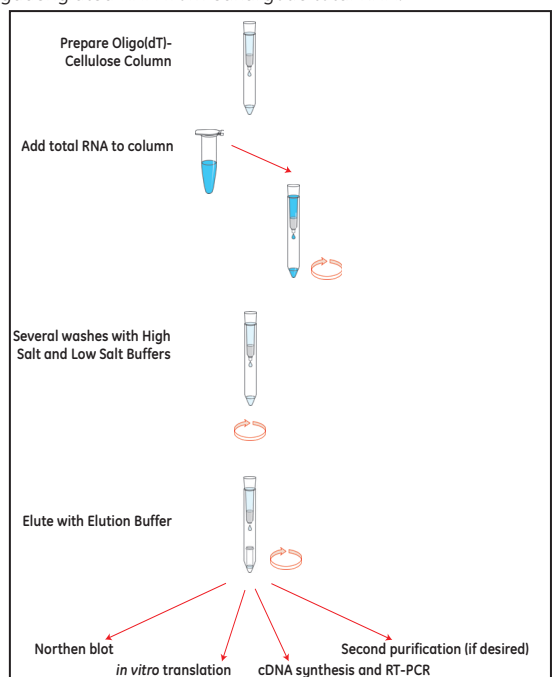


Fig 4.1. Overview of the **mRNA Purification Kit** procedure

Figure 4.1 shows an overview of the RNA isolation procedure using the **mRNA Purification Kit**. It is designed for the rapid purification of

poly(A)+ RNA from eukaryotic total RNA. The key feature of the **mRNA Purification Kit** is its use of spin columns for the affinity purification of poly(A)+ RNA on Oligo(dT)-Cellulose. This approach avoids the slow flow rates and clogged columns that can cause problems during conventional chromatography on Oligo(dT)-Cellulose. These columns are used in a specially developed procedure designed to maximize recovery of poly(A)+ RNA.

4.2. Kit specifications

The starting point for this procedure is total RNA extracted from eukaryotic cells or tissue. For the preparation of this total RNA, we recommend one of the RNA extraction kits from GE Healthcare (see Section 9 for Related Products). RNA isolated with these kits can be applied directly to an Oligo(dT)-Cellulose spin column with no intermediate precipitation. Alternatively, a procedure to isolate total RNA is provided in the Appendix (see Section 8.5).

For optimum results, each total RNA sample should be processed through two successive Oligo(dT)-Cellulose columns. The sample is first heat denatured, then adjusted to an appropriate salt concentration (0.5 M NaCl) using Sample Buffer, and applied to the first pre-packed spin column. Unbound RNA is removed by several washes with High Salt Buffer (0.5 M NaCl) followed by Low Salt Buffer (0.1 M NaCl). The poly(A)+ RNA is then recovered by elution with warm Elution Buffer. The washing and elution steps are all performed very quickly by low speed centrifugation of the column. Approximately 50% of the RNA recovered from the first spin column is poly(A)+ RNA, and in some cases, further purification may not be necessary. However, a second round of spin column chromatography will typically increase the proportion of poly(A)+ RNA to >90% of the sample, with some reduction in final yield (see Section 7.1). This kit includes sufficient columns and buffers for two rounds of chromatography for each RNA sample.

Table 4.1. mRNA Purification Kit Technical Specifications at a Glance

Metric	Specification
Sample size	Total RNA or 25 mg - 1 g of tissue
Elution volume	1000 µl
Binding capacity	25 µg poly(A)+ RNA
% poly(A)+ RNA in the total RNA eluate	50% with one wash step >90% with two wash steps
RNA purity	A_{260}/A_{280}:1.8 - 2.2
Time/Prep	45 min/purification

The poly(A)+ RNA from the final column will often be sufficiently concentrated for immediate use. For those cases where further concentration is required, the kit includes Glycogen Solution and instructions for its use in the quantitative precipitation of small amounts of RNA (see Section 7.2).

5. Preparation of working solutions

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

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 (for optional total RNA preparation)

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. 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. Cesium Trifluoroacetate (CsTFA) (for optional total RNA preparation)

Density CsTFA of 1.51 ± 0.01 g/ml in 0.1 M EDTA, pH7.0

To make 100 ml of this solution, mix 51/(p-1) ml of product number 17-0847-02 (where p = density of CsTFA as received) with 40 ml of 0.25 M EDTA, pH 8.0, and 60-[51/p-1) ml of RNase-free water.

6. Handling, preparation, and storage of starting materials

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

This protocol starts with total RNA and we recommend using illustra™ RNAspin RNA Isolation Kits (see Section 9) to extract total RNA. Alternatively, a basic total RNA isolation protocol is provided in Section 8.5.

7. Protocols

7.1. mRNA purification

Each purification column must be pre-equilibrated before use.

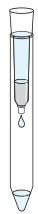
This procedure describes a two column purification, starting with total RNA from 25 mg to 1 g of cells or tissue. The second column can be omitted, in which case, the yield of poly(A)+ RNA will be higher (typically by about 33%), but the relative poly(A)+ purity will be lower (about 50% versus >90%).

Allow 45 minutes for each round of purification. As described in the protocol below, each column must be pre-equilibrated before use by allowing two washes of High Salt Buffer to drain through by gravity. During these washes, prepare the RNA sample for loading. As mentioned previously, a procedure to isolate total RNA is provided in Section 8.5.

1. Column equilibration
Resuspend the **Oligo(dT)-Cellulose** by inverting the column **several times** (with both ends capped)**.**

Invert column several times

2. Storage buffer removal
Remove the top closure followed by the bottom closure. Place the column upright in a 15 ml centrifuge tube and allow the storage buffer to drain through the column.



Drain storage buffer from column

3. High Salt Buffer Wash

Add **1 ml of High Salt Buffer** and allow it to drain through the column by gravity. Repeat this wash step.

After the 2nd wash, discard the flow-through and place the column back in the 15 ml tube.

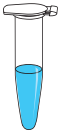
+ 1 ml High Salt Buffer
Let drain
Repeat wash
Discard flow-through

4. RNA preparation

Heat sample at **65°C** for **5 min**.

If starting with a precipitated sample, make sure that the RNA is completely dissolved.

On ice, add **0.2 ml Sample Buffer** and mix gently.



65°C, 5 min
Cool on ice
+ 0.2 ml Sample Buffer

Prewarm Elution Buffer to 65°C.

5. RNA purification

Each column has the capacity to bind approximately 25 µg of poly(A)+ RNA. Assuming that 2% of total RNA is polyadenylated, a maximum of 1.25 mg of total RNA (approximately 31 A_{260} units) should be applied to the column.

Apply sample to the top of the cellulose bed in the column and

Apply sample to column and let soak-in by gravity

allow it to flow into the cellulose bed by gravity.

Centrifuge the column at **350 x *g* for 2 min.**

Spin 2 min, 350 x *g*

6. Washes

<u>High Salt Wash</u>

Add **0.25 ml High Salt Buffer** to the column. Centrifuge for **2 min at 350 x *g*.** Discard the flow-through and return the column to the 15 ml tube.

+ 0.25 ml High Salt Buffer

Repeat wash using **0.25 ml High Salt Buffer** and centrifuge.

Spin 2 min, 350 x *g*

<u>Low Salt Wash</u>

Add **0.25 ml of Low Salt Buffer** to the column. Centrifuge for **2 min at 350 x *g***.

Repeat wash

+ 0.25 ml Low Salt Buffer

Repeat this wash step **twice** more.

Discard the flow-through after the 3rd wash step.

Spin 2 min, 350 x *g*

Repeat twice

Discard flow-through

7. Elution

Place a sterile 1.5 ml microcentrifuge tube inside the 15 ml tube to collect subsequent effluents from the column. Replace the column with its lower end inside the microcentrifuge tube.
To elute bound poly(A)+ RNA, wash the column **4 times** with successive **0.25 ml** aliquots of **Elution Buffer, prewarmed to 65°C**. Centrifuge at **350 x *g* for 2 min** after each application and collect entire eluate in the same sterile tube.

Remove spin column from the centrifuge tube. Using sterile forceps, recover the microcentrifuge tube containing the column eluate.

+ 0.25 ml pre-warmed 65°C Elution Buffer

Spin 2 min, 350 x *g*

Repeat 3 more times

Eluted RNA should be immediately placed on ice to prevent potential degradation. Keep at -20°C and -80°C for short- and long-term storage, respectively.

If the eluate is to be subjected to another round of purification, repeat the mRNA purification procedure, treating the eluate as though it were unfractionated total RNA in Elution Buffer. That is, heat denature the entire eluate, cool and then add **0.2 ml of Sample Buffer** and apply it to a second column.

In many cases, it should be possible to use the RNA eluted from the second column directly, without further concentration. If a more concentrated sample is required, refer to Section 7.2.

7.2. Support protocol mRNA purification kit: concentration of purified mRNA

Transfer purified mRNA to a sterile 15 ml Corex-type centrifuge tube. Add **100 µl of Sample Buffer, 10 µl of Glycogen Solution** and **2.5 ml of ice-cold ethanol**. Mix and **chill at -15 to -30°C** for at least **2 hr.** Centrifuge at **4°C for 10 min at 12 000 x *g*.**

If the mRNA will not be used immediately, it should be stored frozen at -80°C, preferably as an ethanol precipitate.

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 NanoDrop® 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 (3):

$[RNA] = A_{260} \times D \times 40$ µg/ml

D = final dilution factor (in the simplest case above, this would be 1).

8.2. Composition of kit components

Table 8.1. Composition of Kit Components

Oligo(dT)-Cellulose columns	Suspended in storage buffer containing 0.15% Kathon™ CG/1CP Biocide
High Salt Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.5 M NaCl
Sample Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 3 M NaCl
Low Salt Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.1 M NaCl
Elution Buffer	10 mM Tris-HCl (pH 7.4), 1 mM EDTA
Glycogen Solution	5-10 mg/ml Glycogen

8.3. References

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

2. Okayama, H. *et al*., *Methods Enzymol*., **154**, 3, (1987).

3. 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: The column drains slowly

Possible cause	Suggestions
Air bubble in frit	• Typically, a sample should drain through a column in 15-30 minutes. If it takes longer than 30 minutes, there may be an air bubble stuck in the frit of the column or the samples may be too viscous. If a sample does not drain, push the top cap back on the column. This will usually apply sufficient pressure to push any air bubbles out of the frit. To prevent air from being pulled back into the column, replace the bottom cap onto the tip of the column before removing the top cap. After the top cap has been removed, the bottom cap can be removed and the column should drip normally.
Viscosity of the sample	• If the viscosity of the sample is a problem, the column may drain slowly and then stop. The caps can be placed on the column and

Possible cause	Suggestions
Viscosity of the sample	the column gently rocked for 10-15 minutes to allow the sample to bind. The column can be centrifuged to clear it of the unbound material. Proceed with the protocol as written.

Problem: Low yield

Possible cause	Suggestions
Sample material	• Typically, low yield is not a problem as long as sufficient RNA was loaded initially on the column. Insufficient amounts of RNA may be loaded if the extraction method used to purify the total RNA from cells was ineffective. 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 total RNA prepared from 1 g of cells or tissue (approximately 1.25 mg or 31 A_{260} units) 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 and use RNAspin Midi Filter units (see Section 9. Related products) for easy homogenization of disrupted starting material.

8.5. Isolation of total RNA

If extracting RNA from tissue rather than cultured cells, dice the tissue into small cubes prior to isolating total RNA.

Protocol requires the use of Guanidinium Thiocyanate and potentially Cesium Trifluoroacetate

1. Homogenization of sample

Add **18 ml of 5.5 M Guanidinium Thiocyanate (GTC)** solution to each gram of cells or diced tissue.

Disrupt the cells or tissue at room temperature with either manual or mechanical homogenization. Avoid the generation of excess heat or foam.

2. Clarify crude lysate

Transfer the **lysate** to a sterile 50 ml conical centrifuge tube. Centrifuge at **1 500 x *g* for 5 min**.

3. Shear genomic DNA

Transfer the **clarified lysate** to a sterile beaker taking care not to disturb the pelleted material.
Shear the DNA by passing the lysate through a 16-18 gauge needle attached to a 50 ml syringe until the viscosity decreases; this normally takes **10 passes**.

4. Separation of cellular debris

Centrifuge the **sheared lysate** at **5 000 x *g* for 20 min at 15°C** to remove the cellular debris. Decant the supernatant to a clean 50 ml conical tube taking care not to dislodge pelleted debris.

If necessary, adjust the total volume of the supernatant to desired sample loading volume using 5.5 M GTC solution.

5. RNA Separation
Select the number of centrifuge tubes required (see Table 8.6), and prepare appropriately sized cushions of **cesium trifluoroacetate (CsTFA)** solution in these centrifuge tubes. Overlay **aliquots of the sample** onto these cushions.

Centrifuge at **125 000 x *g*** in a swinging bucket rotor for **24 hr at 15°C**. Under these conditions, RNA will pellet on the bottom of the tube, and DNA will collect in a band in the lower third of the gradient that forms.

After centrifugation, carefully aspirate off the bulk of the liquid in each tube, stopping as soon as the DNA band has been removed (or if the DNA band is not visible, stopping approximately 1 cm from the bottom). Decant the remaining liquid, taking care not to disturb the RNA pellet. Invert the tubes and allow them to drain on a paper towel for **5 min** at **room temperature**.

6. Re-hydration of RNA
Redissolve the RNA pellet in the tube bottoms using **two 250 µl** aliquots of **sterile TE buffer** with repeated pipetting. Transfer the RNA solution to a sterile microcentrifuge tube and vortex thoroughly. Heat at **65°C for 10 min**. Vortex again.

Centrifuge briefly to remove any insoluble material. Transfer supernatant to a sterile microcentrifuge tube.

This total RNA can be used immediately for the purification of mRNA. Otherwise, store it at -80°C, preferably as an ethanol precipitate.

8.6. Ultracentrifuge information (for optional total RNA isolation protocol)

Table 8.6. Selection of appropriate volumes, tubes, rotors and speeds

Rotor Type*	SW 50.1	SW 28.1	SW 28
Minimum mass of tissue per tube (mg)	25	100	400
Maximum mass of tissue per tube (mg)	140	450	1000
CsTFA per tube (ml)	2.7	8.5	19.0
Sample loading per tube (ml)	2.5	8.0	18.0
rpm for 125 000 × *g*	31 000	25 000	25 000

*The figures in this table relate to Beckman rotors and centrifuges (Beckman Coulter, Inc., Fullerton, CA), but should be readily convertible for equipment from other manufacturers.

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™ *Micro* mRNA Purification Kit	27-9255-01
QuickPrep mRNA Purification Kit	27-9254-01
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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mRNA Purification Kit
Protocol reminder card

27-9258-01

To isolate mRNA from eukaryotic total RNA

1. Column equilibration

Invert column several times

2. Storage buffer removal

Drain storage buffer from column

3. High Salt Wash

+ 1 ml High Salt Buffer

Let drain

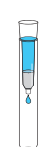
Repeat wash

Discard flow-through

4. RNA preparation

65°C, 5 min
Cool on ice
+ 0.2 ml Sample Buffer
Mix

5. RNA purification

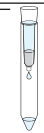


Apply sample to column and soak-in by gravity

Spin 2 min, 350 x *g*

6. Washes

High Salt Washes



+ 0.25 ml High Salt Buffer

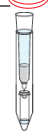
Spin 2 min, 350 x *g*
Repeat wash

Low Salt Washes

+ 0.25 ml Low Salt Buffer

Spin 2 min, 350 x *g*
Discard flow-through
Repeat twice

7. Elution



+ 0.25 ml prewarmed 65°C Elution Buffer

Spin 2 min, 350 x g
Repeat 3 times

Remove spin column from the centrifuge tube.
Eluted RNA should be immediately placed on ice. Keep at -20°C and -80°C for short- and long-term storage, respectively.

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