



Purification of poly(A) RNA

User manual

NucleoTrap[®] mRNA

May 2007/Rev. 03

Protocol-at-a-glance (Rev. 03)

Purification of poly(A) mRNA



Mini/Midi

NucleoTrap® mRNA

<p>1 Adjust binding conditions</p>	<p>Proceed from a total RNA pellet</p> <table border="1"> <tbody> <tr> <td>100-500 µg</td> <td>500 µl RM1</td> </tr> <tr> <td>500-1000 µg</td> <td>1000 µl RM1</td> </tr> </tbody> </table> <p>Proceed from a total RNA solution</p> <table border="1"> <tbody> <tr> <td>200-500 µl</td> <td>1 vol RM0</td> </tr> </tbody> </table>	100-500 µg	500 µl RM1	500-1000 µg	1000 µl RM1	200-500 µl	1 vol RM0
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<p>2 Bind poly(A) RNA</p>	<p>15 µl oligo(dT) latex beads suspension per 100 µg total RNA</p> <p>68°C 5 min</p> <p>RT 10 min invert every 2 min</p> <p>15 s 2,000 x g</p> <p>2 min 11,000 x g</p>						
<p>3 Washing</p>	<p>Discard supernatant from step 2 and resuspend pellet in washing buffer RM2. Transfer the oligo(dT) latex beads suspension onto the NucleoTrap® microfilter.</p> <table border="1"> <tbody> <tr> <td>1st wash</td> <td>600 µl RM2</td> </tr> <tr> <td>2nd wash</td> <td>500 µl RM3</td> </tr> <tr> <td>3rd wash</td> <td>500 µl RM3</td> </tr> </tbody> </table> <p>15 s 2,000 x g</p> <p>2 min 11,000 x g</p>	1st wash	600 µl RM2	2nd wash	500 µl RM3	3rd wash	500 µl RM3
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2nd wash	500 µl RM3						
3rd wash	500 µl RM3						
<p>4 Dry oligo(dT) latex beads</p>	<p>1 min 11,000 x g</p>						
<p>5 Elute highly pure RNA</p>	<p>20 µl H₂O (RNase free) per 10 µl oligo(dT) latex beads</p> <p>68°C 7 min</p> <p>1 min 11,000 x g</p>						

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1 Kit contents

NucleoTrap [®] mRNA		
Cat. No.	12 preps (mini)	12 preps (midi)
	740655	740656
NucleoTrap [®] oligo(dT) latex beads*	480 µl	1800 µl
Buffer RM0	12 ml	12 ml
Buffer RM1	12 ml	12 ml
Buffer RM2	10 ml	20 ml
Buffer RM3	15 ml	15 ml
H ₂ O (RNase-free)	4 ml	8 ml
NucleoTrap [®] microfilter	12	12
Microcentrifuge tubes	24	24
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* For preparation of working solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

NucleoTrap® poly(A) RNA kits are recommended for the isolation and enrichment of poly(A) RNA from a total RNA preparation. Most of eucaryotic mRNA molecules contain relatively long stretches (about 200 residues) of poly(A) at their 3' ends. Thus most mRNAs can be isolated via their poly(A) tracks. The quantity of poly(A) RNA is usually between 1-5% of total cellular RNA. The percentage of poly(A) RNA present depends on cell type, the growth/physiological state of the cell and storage conditions of the cell material. Enrichment of poly(A) RNA is highly recommended for the construction of cDNA libraries or demanding blotting procedures for which a reduction in background signals is desirable e.g. very low abundant transcripts.

2.2 About this User Manual

Experienced users who are performing the isolation of poly(A) RNA using a **NucleoTrap® mRNA** isolation kit may refer to the Protocol-at-a-glance instead of this User Manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure. First-time users are strongly advised to read this User Manual.

2.3 Kit specifications

- **NucleoTrap® mRNA** kits contain latex beads which are covalently modified with oligo(dT) residues. Under high-salt conditions, poly(A) RNA will bind to these beads. As the resulting A-(dT) hybrids can be destabilized under lower ionic strength conditions, poly(A) RNA can be eluted with water or low salt buffer. For the elution of poly(A) RNA we recommend using the supplied H₂O (RNase-free).
- A support protocol contains information for direct purification of poly(A) RNA from cells. In this case, additional equipment and buffers are necessary which are not included in the kit. In general, we recommend purification of poly(A) RNA from total RNA preparations.
- High binding capacity: >5 µg poly(A) RNA / 20 µl oligo(dT) latex beads suspension
- The **NucleoTrap® mRNA mini and midi kits** contain a 50 mg/ml suspension of oligo(dT) latex beads in 10 mM Tris/HCl, 0.1 M NaCl, 0.1% SDS, 0.05% NaN₃ (pH 7.5).

- Both kits are sufficient for 12 poly(A) RNA preparations.
- Precipitated total RNA pellets as well as total RNA solutions can be used as starting material.
- Purified poly(A) RNA from **NucleoTrap® mRNA** kits is ready for use in all downstream applications.
- Poly(A) RNA is not degraded and without DNA contaminations.
- Each **NucleoTrap® mRNA mini preparation** includes 40 µl oligo(dT) latex beads which allow processing of 200-250 µg of total RNA on average and give a maximum yield of 10 µg poly(A) RNA.
- Each **NucleoTrap® mRNA midi preparation** includes 150 µl latex beads which allow processing of up to 1000 µg of total RNA on average and give a maximum yield of 40 µg poly(A) RNA. Both kits are highly flexible and allow appropriate combination of total RNA and latex bead suspension.

Table 1: Kit specifications at a glance

	mRNA (Mini)	mRNA (Midi)
Sample size	up to 250 µg total RNA	up to 1000 µg total RNA
Yield	up to 10 µg	up to 40 µg
Elution volume	20 µl/ 10 µl oligo(dT) latex beads	20 µl/ 10 µl oligo(dT) latex beads
Binding capacity	5 µg poly(A) RNA / 20 µl oligo(dT) latex beads suspension	5 µg poly(A) RNA / 20 µl oligo(dT) latex beads suspension
Time/prep	30 min*	30 min*

*Hands-on time

2.4 Isolation of total RNA

For the purification of high quality intact poly(A) RNA, a critical parameter is the quality of the total RNA. Therefore we recommend to isolate total RNA first. Further information is given in the following tables:

Kits for the isolation of total RNA using NucleoSpin® technology			
	RNA II (Mini)	RNA L (Midi)	RNA Plant (Mini)
Cat. No	740955.20 / 50 / 250	740962.20	740949.20 / 50 / 250
Sample size	up to 5 x 10 ⁶ cells up to 30 mg tissue	up to 5 x 10 ⁷ cells up to 200 mg tissue	up to 100 mg plant tissue or filamentous fungi
Average yield	up to 70 µg	up to 400 µg	up to 70 µg

Kits for the isolation of total RNA using NucleoBond® technology		
	RNA/ DNA 80 (Mini)	RNA/ DNA 400 (Midi)
Cat. No.	740650	740651
Sample size	up to 5 x 10 ⁶ eucaryotic cells up to 0.5 x 10 ⁸ bacterial cells up to 20 mg tissue	up to 2 x 10 ⁷ eucaryotic cells up to 2 x 10 ⁹ bacterial cells up to 100 mg tissue
Average yield	up to 70 µg	up to 400 µg

2.5 Handling, preparation, and storage of starting materials

Eluted total RNA should immediately be put and always kept on ice for optimal stability because almost omnipresent RNases (general lab ware, fingerprints, dust) will degrade RNA. For short-term storage freeze at -20°C , for long-term storage freeze at -70°C .

Optimal RNA stability during long term storage is achieved by storing RNA in a precipitated form. Add 1/10 volume of 3M sodium acetate and 2.5 volume of ethanol (96-100%) to the RNA, mix and store preferably at -20°C to -80°C . Before use, pellet RNA by centrifugation, wash RNA pellet with 70% ethanol and resuspend in H_2O (RNase-free).

3 Storage conditions, preparation of working solutions

Attention:

Buffers RM1, RM2, RM3 and RM0 contain LiCl. Wear gloves and goggles!

- **Oligo(dT) latex beads** should be stored at 4°C upon arrival. All other kit components may be stored at 4°C or room temperature (20-25°C). Storage at 4°C may cause salt precipitation, in which case, buffers should be preheated to 37°C before use. The oligo(dT) latex beads settle to the bottom of the tube. Therefore, the oligo(dT) latex beads suspension should be vortexed moderately before use, to ensure equal distribution.
- All kit components are stable up to one year if stored correctly as described above.

4 Safety instructions – risk and safety phrases

The following components of the NucleoTrap® mRNA kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol	Risk Phrases	Safety Phrases
RM0	Lithium chloride	Substance does not have to be specially labeled as hazardous		
RM1	Lithium chloride	Substance does not have to be specially labeled as hazardous		
RM2	Lithium chloride	Substance does not have to be specially labeled as hazardous		
RM3	Lithium chloride	Substance does not have to be specially labeled as hazardous		

5 Protocols for the isolation of poly(A) RNA

5.1 Poly(A) RNA isolation from total RNA with NucleoTrap[®] mRNA

1 Adjust binding conditions

RNA pellet

Add **500 µl buffer RM1** to a pellet which contains **100-500 µg** total RNA and **1000 µl buffer RM1** to a pellet which contains up to **1000 µg** total RNA. Pipette up and down and vortex well in order to guarantee a good resuspension.



+ 500 µl or
1000 µl RM1

RNA solution

To process a **200-500 µl total RNA sample** (in water, TE buffer or usual reaction buffers) add the **same volume of RM0** binding buffer.

+ 1 vol RM0

2 Bind poly(A) RNA

Resuspend the oligo(dT) latex beads by vortexing. Add **15 µl oligo(dT) latex beads suspension** per **100 µg** total RNA. Mix well and **heat at 68°C for 5 min**. Incubate at **room temperature for 10 min** and invert the tube every 2 min during incubation.



+ 15 µl
oligo(dT) latex
beads
suspension

68°C
5 min

During heat incubation the secondary structure of RNA is denatured. Mixing and subsequent incubation at room temperature are important for efficient binding of poly(A) RNA to the oligo(dT) latex beads.

RT
10 min
invert every
2 min

Centrifuge for **15 s at 2,000 x g**, then for **2 min at 11,000 x g** in a microcentrifuge tube.



15 s
2,000 x g

The high-speed centrifugation step is recommended for obtaining a tight pellet and for minimizing the loss of oligo(dT) latex beads.

2 min
11,000 x g

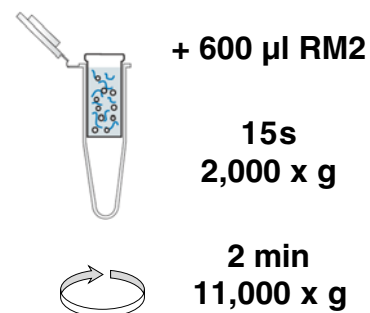
3 Washing

1st wash

Discard supernatant and dissolve pellet completely in **600 µl washing buffer RM2** by pipetting up and down and vortexing.

Dissolve pellet completely until solution becomes „milky“ and no pellet is visible – this step is important for optimal removal of contaminants such as DNA and rRNA.

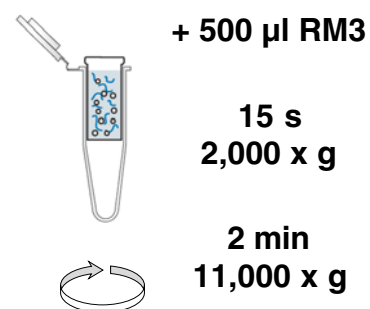
Transfer the oligo(dT) latex bead suspension onto the **NucleoTrap® microfilter** and centrifuge for **15 s at 2,000 x g**, then for **2 min at 11,000 x g**. Discard flow-through. The oligo(dT) latex beads are retained in the filter insert.



*If more than 500 µg total RNA have been used in 1st wash, perform an additional washing step: Add **400 µl washing buffer RM2** to the oligo(dT) latex beads on the NucleoTrap® microfilter. Resuspend oligo(dT) latex beads directly on the NucleoTrap® microfilter by pipetting up and down carefully. Centrifuge for **15 s 2,000 x g** and for **2 min at 11,000 x g**. Discard flow-through. Avoid puncturing the NucleoTrap® microfilter!*

2nd wash

Add **500 µl washing buffer RM3** to the oligo(dT) latex beads and resuspend them directly on the NucleoTrap® microfilter by pipetting up and down carefully. Avoid puncturing the NucleoTrap® microfilter. Centrifuge for **15 s at 2,000 x g**, then for **2 min at 11,000 x g**. Discard flow-through.

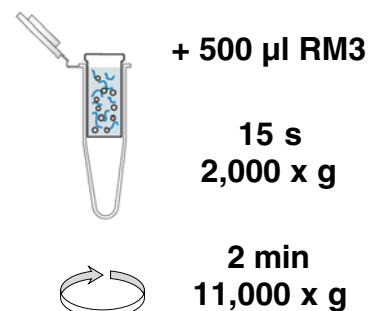


Resuspend oligo(dT) latex beads completely until the solution becomes „milky“ and no pellet is visible. This step is important for the removal of ribosomal RNA. To make the resuspension of the pellet easier, mark the pellet's position after centrifugation, or centrifuge NucleoTrap® microfilter with identical orientation regarding the position of the lid.

3rd wash

Add **500 µl washing buffer RM3** to the oligo(dT) latex beads and resuspend them completely as described in 2nd wash step. Centrifuge for **15 s at 2,000 x g**, then for **2 min at 11,000 x g**. Discard flow-through.

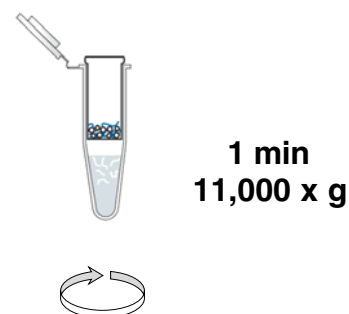
Dissolve pellet completely until the solution becomes „milky“ and no pellet is visible. This step is important for the optimal removal of residual buffer RM2.



4 Dry oligo(dT) latex beads

Centrifuge NucleoTrap[®] microfilter for **1 min at 11,000 x g** to completely remove the washing buffer. Transfer NucleoTrap[®] microfilter to a clean RNase-free 1.5 ml elution tube.

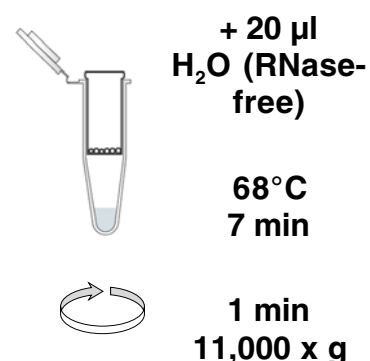
Residual washing buffer may inhibit subsequent reactions.



5 Elute pure poly(A) RNA

Add **20 µl prewarmed (68°C) H₂O (RNase-free)** per **10 µl oligo(dT) latex beads** and resuspend oligo(dT) latex beads completely by pipetting up and down (elution buffer becomes “milky”). Close lids and incubate NucleoTrap[®] microfilter columns at **68°C for 7 min**. Centrifuge for **1 min at 11,000 x g** and collect eluate.

For higher yields: Repeat the elution step and combine eluates - a second elution step will typically result in a 10-20% increased yield but a less concentrated eluate. Transfer combined eluates to a clean 1.5 ml elution tube and store on ice. Subsequent reactions should be performed immediately - if this is not possible, store the eluates at -70°C.



5.2 Direct purification of poly(A) RNA from cells

For direct purification of poly(A) RNA from cells additional equipment and buffers are necessary which are **not included in the NucleoTrap® poly(A) RNA kit**. In general, we recommend purification of poly(A) RNA from total RNA preparations.

Harvest cells

Precipitate cells by centrifugation (Starting material containing approximately 100 µg total RNA should be used, with a maximum of 1×10^8 cells.)

5 min
500 x g

1 Adjust binding conditions

Add 600 µl RMO binding buffer to the sample. To resuspend the cell pellet, pipette up and down and vortex well.

Load lysate onto a **NucleoSpin® Filter** (not included in this kit, see ordering information) and centrifuge for **1 min at 11,000 x g**. Add **600 µl RNase-free water** to the clear flow-through and mix well by vortexing.

Optional: The lysate may be passed alternatively ≥ 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.



+ 600 µl
RMO

2 Bind poly(A) RNA

Resuspend the oligo(dT) latex beads by vortexing. **Add 20 µl oligo(dT) latex beads suspension** per **100 µg total RNA**. Mix well and **heat at 68°C for 5 min**. Incubate at **room temperature for 10 min** and invert the tube every 2 min.

During heat incubation the secondary structure of RNA is denatured. Mixing and subsequent incubation at room temperature are important for efficient binding of poly(A) RNA to the beads.



+ 20 µl
oligo(dT)
latex beads
suspension

68°C
5 min

RT
10 min
invert every
2 min

Centrifuge for **15 s at 2,000 x g**, then for **5 min at 11,000 x g** in a microcentrifuge tube

The high-speed centrifugation step is recommended for obtaining a tight pellet and for minimizing the loss of oligo(dT) latex beads.



15 s
2,000 x g

5 min
11,000 x g

Proceed with the standard protocol step 3 section 4.1.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestion
<p>Low yield and/or degraded poly(A) RNA</p>	<p><i>Washing procedure: washing buffer RM3 was not removed completely during centrifugation</i></p> <ul style="list-style-type: none"> • Prolong the subsequent centrifugation step in order to dry NucleoTrap[®] microfilter and oligo(dT) latex beads. <p><i>Elution procedure – check the following parameters:</i></p> <ul style="list-style-type: none"> • Elution volume too low? • Elution buffer too cold? • Oligo(dT) latex beads completely resuspended in elution buffer? <p><i>poly(A) RNA binding and integrity</i></p> <ul style="list-style-type: none"> • To avoid insufficient binding of poly(A) RNA, check incubation temperature and time during hybridization. • Check integrity of total RNA preparation on a denaturing agarose gel before enrichment of poly(A) RNA. • RNase contamination: clean working place and use RNase-free pipette tips and gloves.
<p>Subsequent reactions failed</p>	<p><i>RM3 washing and removal</i></p> <ul style="list-style-type: none"> • Repeat RM3 washing. Prolong subsequent centrifugation step in order to dry the NucleoTrap[®] microfilter and oligo(dT) latex beads in order to remove any RM3 buffer. <p><i>poly(A) RNA quality verified?</i></p> <ul style="list-style-type: none"> • Process positive controls for subsequent reactions. • Check poly(A) RNA quality by gel electrophoresis or blotting experiments with standards.

Problem	Possible cause and suggestion
Direct isolation of poly(A) RNA from cells failed	<p><i>Insufficient lysis</i></p> <ul style="list-style-type: none"> • Instead of using lysis buffer RM0, alternative buffers for direct poly(A) RNA isolation can be used. For example, 1 M GITC or 0.5 M NaCl with 1% SDS, 5 mM DTT at pH 7-8. Keep in mind that low ionic strength conditions destabilize binding of poly(A) RNA to the oligo(dT) latex beads. Avoid using < 0.1 M salt for binding and washing procedures. Always ensure that all reagents are RNase-free.
	<p><i>Total RNA contains approximately 80% of rRNA</i></p> <ul style="list-style-type: none"> • It is difficult to recover poly(A) RNA which is rRNA free with a single oligo(dT) selection round using the NucleoTrap[®] mRNA kit. A typical poly(A) isolation with NucleoTrap[®] mRNA kit yields poly(A) RNA with a reduced rRNA level acceptable for virtually all molecular biological procedures. If lower rRNA levels are desirable, perform a second selection round with the NucleoTrap[®] mRNA kit.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoTrap [®] mRNA (mini)	740655	12
NucleoTrap [®] mRNA (midi)	740656	12
NucleoSpin [®] Filter	740606	50

6.3 Product use restriction / warranty

NucleoTrap® mRNA kit components were developed, designed, distributed 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 rather the responsibility of the user to verify the use of the **NucleoTrap® mRNA** 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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Last updated 12/2006, Rev. 02