

Total RNA and mRNA isolation directly from various Animal tissue

RNA is the most versatile biological molecule. Obtaining high quality and intact RNA is the first and often the most critical step in performing many fundamental molecular biology experiments, including Northern analysis, nuclease protection assays, RT-PCR, RNA mapping, in vitro translation and cDNA library construction.

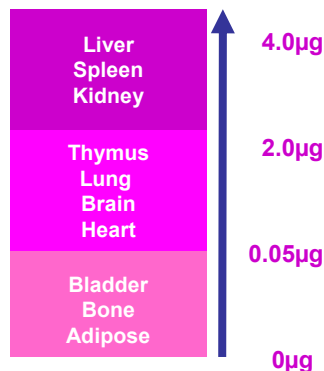
By Frédéric Freund
ADEMTECH SA

Introduction

Tissues disruption is the first step in RNA isolation and one of the most critical steps affecting yield and quality of the isolated RNA. The reason is that tissues contain RNase (ribonucleases). RNase with other intracellular enzymes are normally sequestered in vesicles (lysosomes, peroxysomes...). When these vesicles are disrupted, their content could destroy the RNA.

Tissues disruption need to be fast and thorough. Ademtech has developed a reagent and a kit that enable rapid total and mRNA purification from tissues with a high level of RNase.

Table 1 : Expected total RNA yields from 1 mg of various tissues (µg total RNA/ mg tissue)



Materiel and Methods

Tissue Preparation : Immediately after dissection tissues were frozen and kept at -80°C until using.

Total RNA purification :

Samples were weighted into 50mg aliquots. Each aliquot was disrupted and homogenized with a conical homogenizer for connective tissues (wheaton 1-3ml). The extraction procedure used was strictly the one described in the product protocol. Briefly : 1/ disrupt 50mg of tissues in 1ml of Total RNA isolation Reagent. 2/ Add 0,1ml of chloroform and mix. 3/ Centrifuge and keep the upper aqueous phase. 4/ Precipitate RNAs with isopropanol. 5/ Wash with ethanol 75%. 6/ Dry and recover the total RNA in Water.

mRNA purification :

mRNA purification were performed with the Direct mRNA dembeads purification kit. Samples were weighted into 25mg or 50mg, depending of the tissue. Their were disrupted and homogenized with a conical homogenizer for connective tissues (wheaton 1-3ml). The extraction procedure used was strictly the one described in the product protocol. Briefly: 1/ disrupt tissue samples in 1ml of Lysis Buffer. 2/ Complex the Streptavidin nanoparticles to the biotinylated oligonucleotides (dT)₂₅. 3/ Incubate the complexes with the tissue lysates. 4/ Wash the complexes. 5/ Elute the mRNA.

RT-PCR analysis :

Five microlitres of purified RNA were reverse transcribed using M-MLV RT(-) and poly(dT)₁₅ primer. Then, PCR was performed with ACC (Acetyl-CoA carboxylase) primers on 5µl of cDNA previously formed. Finally, 10µl of each PCR reaction were analysed on a 2% agarose gel in TBE buffer.

Total RNA isolation Reagent

High yields of pure total RNA

Organ	Weight (mg)	Yield (μg)	$A_{260/280}$ ratios	$A_{260/230}$ ratios
Kidney (K)	50	110-120	1,7	2,5
Brain (B)	50	30-40	1,7	2,6
Liver (Li)	50	140-150	1,7	2,5
Lung (Lu)	50	45-55	1,7	2,5
Heart (H)	50	30-40	1,8	2,5
Thymus (Th)	50	90-100	1,7	2,5

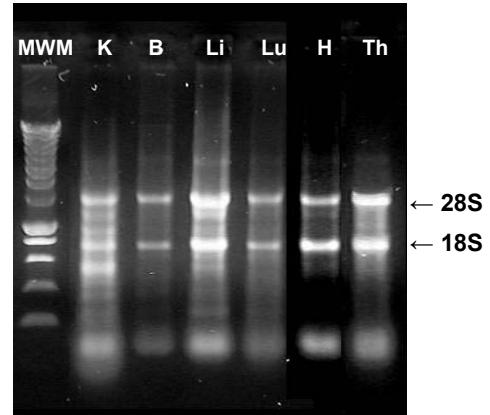


Table 2 : Spectrophotometer analysis of RNA tissues samples

Table 2 presents the yield obtained from a variety of rat organs. Yields can vary because of extrinsic factors such as the metabolite state of the animal and the type of organ. The isolated total RNA all display $A_{260/280}$ ratios indicating efficient removal of proteins. The $A_{260/230}$ ratios indicate no phenol or ethanol residual contaminations. The quality of total RNA was assessed by agarose gel electrophoresis (Figure 1). Furthermore the RNA was not degraded, and was demonstrated by visualizing the two ribosomal bands. The RNA prepared from several tissues was free of genomic DNA.

Figure 1 : None-denaturing agarose gel analysis (1% agarose in TBE buffer containing 0,7 $\mu\text{g}/\text{ml}$ ethidium bromide) of RNA samples prepared with the Total RNA isolation Reagent.

Applications of total RNA

Total RNA is suitable for many molecular biology applications, including RT-PCR analysis, Northern Blot, *in vitro* translation or mRNA purification. Total RNA isolation Reagent was able to purify RNA in high yield and quality from all the animal tissues. As shown in Figure 2, RNA purified exhibit an excellent integrity and was suitable for RT-PCR.

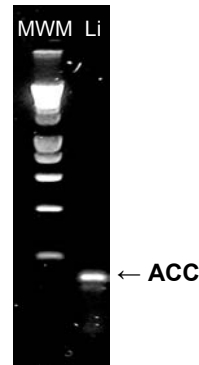


Figure 2 : RT-PCR of ACC transcript from total RNA isolated using the Total RNA isolation Reagent protocol. ACC is an Acetyl-CoA carboxylase having a great importance for lipid metabolism.

Total RNA isolation Reagent

Benefits :

- Simple procedure
- Isolate high quality, intact RNA
- Free of genomic DNA
- Reproducible high yields
- Convenient for various animal tissues

Ordering information :

Size: 200ml

Code number : 10603

Direct mRNA Adembeads purification kit

Principle of the Direct mRNA Adembeads purification kit

The Direct mRNA Adembeads purification kit combine the affinity of biotin to streptavidin with biomagnetic separation. The oligonucleotides (dT) is linked to biotin. Biotinylated Oligo (dT) are linked to the surface of the Streptavidin superparamagnetic nanoparticles. The isolation of mRNA start with the addition of [Streptavidin nanoparticles-biotinylated oligo (dT)] complexes directly to tissue lysate. The oligo (dT) binds the 3' poly (A) tail present in most mature eukaryotic mRNA species. Figure 1 illustrates the procedure used for isolation of mRNA from animal tissues. The protocol require minimal handling time in as little 20 minutes.

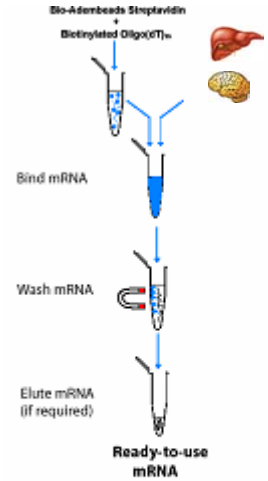
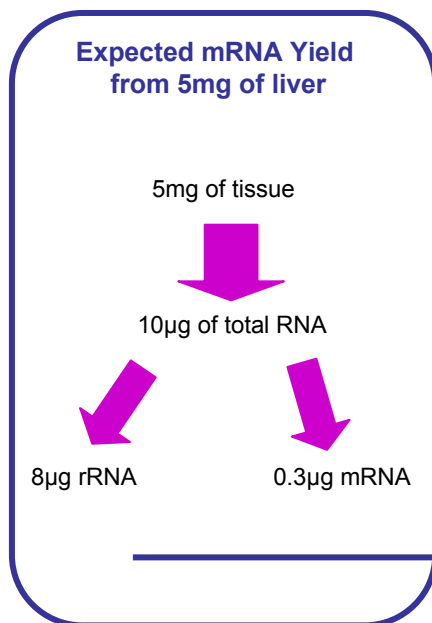


Figure 3 : Animal tissue mRNA isolation using Direct mRNA Adembeads purification kit.

High yields of pure mRNA

Organ	Weight (mg)	Volume of Direct mRNA Adembeads reagent	A _{260/280} ratios	Yield (µg)
Liver (Li)	25	200µg SA-Adembeads	2	1-2.5
		16pmol oligo d(T) probe		
		1ml Lysis Buffer		
Lung (Lu)	50	400µg SA-Adembeads	2.2	0.5-1
		32pmol oligo d(T) probe		
		1ml Lysis Buffer		
Brain (B)	50	400µg SA-Adembeads	2.15	0.5-1
		32pmol oligo d(T) probe		
		1ml Lysis Buffer		

Table 3 : Spectrophotometer analysis of mRNA tissues samples



The Direct mRNA Adembeads purification kit offers significant benefits over conventional mRNA isolation methods. Table 3 provides a usage table and demonstrates the flexibility in system. The direct mRNA can be used directly from various tissue samples. The yield of mRNA were in the range of 0.5-3% of the Total RNA depending on the tissue. High yields of pure mRNA are obtained with the Direct mRNA Adembeads kit, without loss of mRNA that typically occurs during the organic extractions. The isolated mRNA all display A_{260/280} ratios indicating efficient removal of proteins.

Direct mRNA dembeads purification kit

High quality of mRNA

The quality of mRNA was assessed by agarose gel electrophoresis. Low rRNA contamination of the mRNA extracts was observed with the Direct mRNA dembeads purification kit.

Application of pure mRNA

Purified mRNA with Direct mRNA dembeads purification kit is suitable for many molecular biology applications, including RT-PCR analysis, in vitro translation, cDNA synthesis and Northern Blot. Direct mRNA dembeads purification kit was able to purify mRNA in high yield and quality from all the animal tissues and as shown in Figure 6, purified mRNA exhibit excellent integrity and was suitable for RT-PCR.

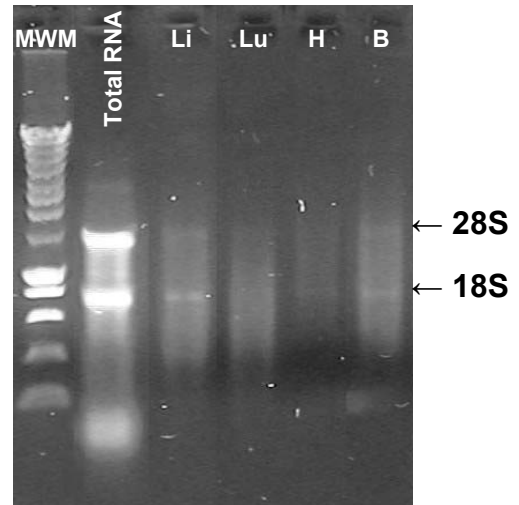


Figure 5 : None-denaturing agarose gel analysis (1% agarose in TBE buffer containing 0,7mg/ml ethidium bromide) of mRNA samples prepared with the Direct mRNA dembeads purification kit.

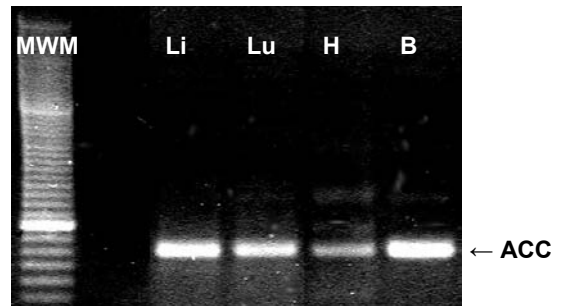


Figure 6 : RT-PCR of ACC transcripts from mRNA isolated using the Direct mRNA dembeads purification kit protocol. ACC is an Acetyl-CoA carboxylase.

Direct mRNA dembeads Purification kit benefits

- Fast system less than 20 min
- High yield
- High purity
- No mRNA degradation
- No DNA contamination
- Easy to handle
- Detect low level of mRNA
- Low rRNA contamination
- Convenient for various samples

From cells

From whole Blood

From animal tissues

From plant tissues

Product	Size	Code number
Direct mRNA dembeads purification kit	30 isolations each from up to 25mg of liver or 15 isolations from up to 50mg of heart	06021