

RNA Subcellular Isolation Kit

(version A1)

Catalog No. 25501

Active Motif North America

1914 Palomar Oaks Way, Suite 150

Carlsbad, California 92008, USA

Toll free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

Active Motif Europe

Avenue Reine Astrid, 92

B-1310 La Hulpe, Belgium

UK Free Phone: 0800 169 31 47

France Free Phone: 0800 90 99 79

Germany Free Phone: 0800 181 99 10

Telephone: +32 (0)2 653 0001

Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor

2-21 Ageba-Cho, Shinjuku-Ku

Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638

Fax: +81 3 5261 8733

Active Motif China

787 Kangqiao Road

Building 10, Suite 202, Pudong District

Shanghai, 201315, China

Telephone: (86)-21-20926090

Hotline: 400-018-8123

Copyright 2015 Active Motif, Inc.

Information in this manual is subject to change without notice and does not constitute a commitment on the part of Active Motif, Inc. It is supplied on an “as is” basis without any warranty of any kind, either explicit or implied. Information may be changed or updated in this manual at any time.

This documentation may not be copied, transferred, reproduced, disclosed, or duplicated, in whole or in part, without the prior written consent of Active Motif, Inc. This documentation is proprietary information and protected by the copyright laws of the United States and international treaties.

The manufacturer of this documentation is Active Motif, Inc.

© 2015 Active Motif, Inc., 1914 Palomar Oaks Way, Suite 150; Carlsbad, CA 92008. All rights reserved.

All trademarks, trade names, service marks or logos referenced herein belong to their respective companies.

TABLE OF CONTENTS	Page
Overview	1
Flow Chart of Process	2
Kit Performance and Benefits	3
RNA Subcellular Isolation Kit	3
Kit Components and Storage	5
Additional Materials Required	5
Protocols	
Buffer Preparation and Recommendations	6
Assay Protocol	7
Section A: Preparation of Cultured Cells	7
Section B: Preparation of Fresh Tissue	8
Section C: Preparation of Frozen Tissue	9
Section D: Nuclear & Cytoplasmic RNA Isolation	9
Section E: Total RNA Isolation	10
Section F: RNA Quantification	11
Section G: Downstream Analysis	12
Section H: Data Normalization	13
Appendix	
Section K. Troubleshooting Guide	14
Section L. Related Products	15
Technical Services	16

Overview

The RNA Subcellular Isolation Kit offers an efficient method to isolate RNA from different cellular fractions using cultured cells or fresh tissues. Unlike most RNA preparation methods that isolate only total RNA, Active Motif's RNA Subcellular Isolation Kit enables purification of large and small RNAs from both nuclear and cytoplasmic fractions without cross contamination or the use of phenolic compounds. Additionally, total RNA can be isolated from whole cells and frozen tissue.

Understanding the transcriptome is a complex process. RNA molecules are present at different levels of maturation and processing within a cell at any given time, which can complicate and bias downstream analysis. In some instances, it may be advantageous to identify splice junctions and measure gene expression levels without the background of intronic RNAs from the nucleus. Conversely, it may be desirable to study chromatin-associated RNA or nuclear RNA maturation without the influence of mature RNAs from the cytoplasm. Finally, given the diversity of ncRNAs, understanding RNA localization may help to inform about RNA function. Active Motif's RNA Subcellular Isolation Kit provides a solution to these challenges by enabling the isolation of RNA from specific cellular fractions and making it easier to analyze the RNA of interest or to determine RNA localization.

The RNA Subcellular Isolation method works by lysing cells or tissue samples in complete lysis buffer. The lysate is then separated by centrifugation, with the supernatant containing cytoplasmic RNA and the pellet containing nuclear RNA. A guanidine-based buffer and ethanol are added to each RNA fraction before being loaded onto spin-columns. The column binds the RNA allowing any contaminating protein to flow through the membrane or remain unbound. Following a wash step, the RNA fractions are eluted and quantified. The purified RNA is suitable for use in a variety of downstream applications, including reverse transcription PCR (RT-qPCR), Northern blotting and RNA-seq.

The RNA Subcellular Isolation Kit includes enough reagents to perform 15 cytoplasmic and 15 nuclear preparations, or 30 total RNA purifications.

product	format	catalog no.
RNA Subcellular Isolation Kit	30 rxns	25501
DNase I Treatment Kit	30 rxns	25502

Flow Chart of Process

Subcellular Isolation

Cultured Cells or Fresh Tissue

Add Lysis Buffer

Incubate on ice 10-20 min.



Centrifuge to separate cell fractions

Supernatant containing cytoplasmic RNA

Pellet containing nuclear RNA

Add Buffer G
Add 70% Ethanol

Add Buffer G
Add 70% Ethanol



Load Spin Column



Load Spin Column



Wash



Wash



Elute



Elute

Total RNA

Cultured Cells, Fresh or Frozen Tissue



Add Buffer G
Add 70% Ethanol



Load Spin Column



Wash



Elute

Kit Performance and Benefits

The RNA Subcellular Isolation Kit is designed to isolate nuclear and cytoplasmic RNA from cultured cells and fresh tissue, or total RNA from cultured cells and frozen tissue.

Maximum Column Binding Capacity	40 µg
Maximum Column Loading Volume	750 µl
Size of purified RNA	RNA between the sizes of 75 nt to 7,000 nt
Maximum Amount of Starting Material Mammalian Cells	4 x 10 ⁶ cells (Cytoplasmic and Nuclear RNA) 2 x 10 ⁶ cells (Total RNA)
Mammalian Tissues	15 mg
Assay Time	2 hours
Average Yields HeLa Cytoplasmic RNA HeLa Nuclear RNA HeLa Total RNA	See page 7 for more guidance on cell numbers and RNA yields -12-25 µg at 250-500 ng/µl from 2 x 10 ⁶ cells -12-25 µg at 250-500 ng/µl from 2 x 10 ⁶ cells -25-45 µg at 500-900 ng/µl from 2 x 10 ⁶ cells

RNA Subcellular Isolation Kit

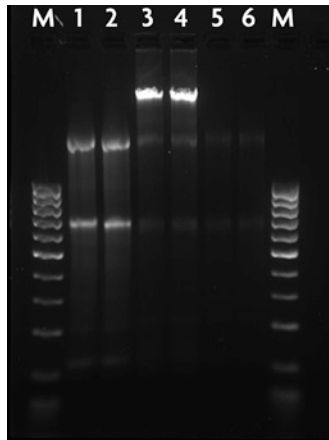


Figure 1: Agarose gel of nuclear and cytoplasmic RNA fractions isolated with the RNA Subcellular Isolation Kit.

RNA was isolated as nuclear and cytoplasmic fractions using the RNA Subcellular Isolation Kit. Following purification, half of the nuclear RNA was treated with DNase I to remove genomic DNA contamination using the DNase I Treatment Kit (Active Motif Catalog No. 25502). 1 µg of each RNA fraction was loaded onto a 1.5% agarose gel for analysis..

Lanes M = 100 bp DNA ladder

Lanes 1-2 = Cytoplasmic RNA

Lanes 3-4 = Nuclear RNA before DNase I treatment

Lanes 5-6 = Nuclear RNA after DNase I treatment

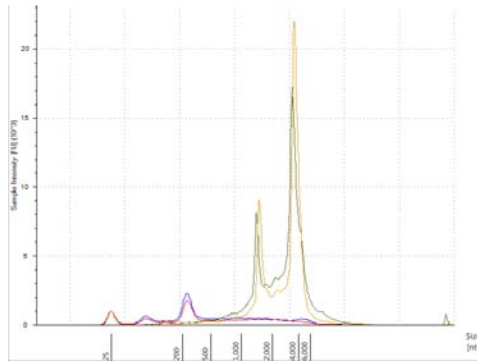


Figure 2: Cytoplasmic RNA trace pre- and post ribosomal RNA (rRNA) depletion.

Cytoplasmic RNA was isolated from HeLa cells using the RNA Subcellular Isolation Kit. TapeStation analysis was performed to analyze the recovered RNA before and after rRNA depletion. The yellow and green traces represent two cytoplasmic samples pre-rRNA depletion. The two large peaks represent the 18S and 28S rRNA. The blue and red traces represent the same samples after rRNA depletion. The 18S and 28S peaks are no longer present. The size of the purified RNA spans from 75 nucleotides to 7,000 nucleotides.

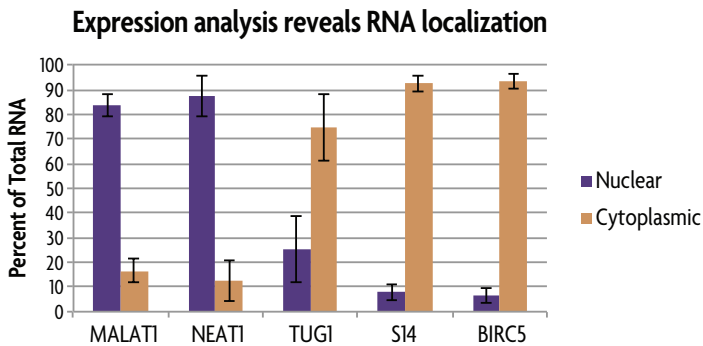


Figure 3: Subcellular RNA isolation to identify RNA localization.

Nuclear, cytoplasmic and total RNA were isolated from HeLa cells using the RNA Subcellular Isolation Kit. Following purification and DNase I treatment of the nuclear and total RNA fraction with Active Motif's DNase I Treatment Kit (Catalog No. 25502) purified RNA was subjected to reverse transcription and qPCR. Each subcellular fraction was plotted as a percentage of total RNA. The data shows the localization of various RNAs. MALAT1, NEAT1 and TUG1 are lncRNAs. Their expression analysis data is consistent with published RNA FISH data for the same RNAs and cell type. Both S14, which is a ribosomal RNA, and BIRC5, a mRNA, are expected to localize within the cytoplasm as demonstrated above.

RNA Subcellular Isolation Kit Components and Storage

RNA Subcellular Isolation Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
1 M DTT	100 μ l	-20°C
10X PBS	120 ml	4°C
Lysis Buffer AM7	5 ml	4°C
Buffer G	20 ml	4°C
Purification Columns	30 ea	4°C
DEPC Water	10 ml	RT

Additional materials required

- Conical tubes and centrifuge
- 70% Ethanol
- β -mercaptoethanol
- RNase-free microcentrifuge tubes and microcentrifuge
- Vortex set to highest setting
- Ice bucket and ice
- Nanodrop, Qubit or similar device for RNA quantification
- Sample material for RNA analysis (cultured cells, fresh or frozen tissue)
- Trypsin (cultured cell samples)
- Hemacytometer (cultured cell samples)
- Microscope (cultured cell samples)
- Razor blades (fresh and frozen tissue samples)
- Analytical balance (fresh and frozen tissue samples)
- Syringes with small (27-29 gauge), medium (21-23 gauge) and large (15-17 gauge) gauge needles (fresh tissue samples)
- Mortar and pestle (frozen tissue samples)
- Liquid nitrogen (frozen tissue samples)
- Dry ice (frozen tissue samples)
- (Optional) DNase I Treatment Kit (Active Motif, Catalog No. 25502)

Protocols

Buffer Preparation and Recommendations

10X PBS

Prepare a 1X PBS by diluting the 10X PBS in sterile water. For every 10 ml needed, dilute 1 ml 10X PBS into 9 ml sterile water.

Preparation of Complete Lysis Buffer

Prepare the amount of Complete Lysis Buffer required for the assay by adding 5 μ l of the provided 1 M DTT per 1 ml Lysis Buffer AM7 (see the Quick Chart for Preparing Buffers in this section). Mix well by pipetting. After use, discard remaining Complete Lysis Buffer.

Preparation of Complete Buffer G

Buffer G contains salts that will precipitate out of solution during cold temperature storage. We recommend placing the buffer at room temperature 1 hour prior to use to allow the solution to warm up. Ensure that the salts are fully dissolved into solution before use by vortexing at room temperature. Prepare the amount of Complete Buffer G required for the assay. Transfer the necessary amount of Buffer G to a clean container. Add 10 μ l of β -mercaptoethanol per 1 ml Buffer G (see the Quick Chart for Preparing Buffers in this section). Mix well by vortexing. Keep the buffer at room temperature during the assay. After use, discard remaining Complete Buffer G.

Preparation of Wash Buffer

Prepare the amount of Wash Buffer required for the assay. Transfer the necessary amount of Buffer G (without β -mercaptoethanol) for the wash steps into a clean container. Add an equal volume of 70% ethanol (e.g. Add 5 ml 70% ethanol to 5 ml Buffer G). Invert repeatedly. The buffer can be stored at room temperature after the addition of ethanol.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	Total RNA For 1 rxn	Nuc & Cyto RNA For 1 rxn each	Total, Nuc & Cyto RNA For 1 rxn each
Complete Lysis Buffer	1 M DTT	–	0.6 μ l	0.6 μ l
	Lysis Buffer AM7	–	120 μ l	120 μ l
	TOTAL REQUIRED	–	120 μl	120 μl
Complete Buffer G	β -mercaptoethanol	3.5 μ l	6 μ l	10 μ l
	Buffer G	346.5 μ l	594 μ l	990 μ l
	TOTAL REQUIRED	350 μl	600 μl	1 ml
Wash Buffer	70% Ethanol	300 μ l	600 μ l	1 ml
	Buffer G	300 μ l	600 μ l	1 ml
	TOTAL REQUIRED	600 μl	1.2 ml	2 ml
70% Ethanol	TOTAL REQUIRED	1.25 ml	2.65 ml	4 ml
DEPC Water	TOTAL REQUIRED	50 μl	100 μl	150 μl

Assay Protocol

Read the entire protocol before use. Ensure work space and lab supplies are free from RNases. Wear gloves at all times.

The RNA Subcellular Isolation Kit is designed to purify nuclear and cytoplasmic RNA from cultured cells and fresh tissue, or to isolate total RNA from whole cells, fresh or frozen tissue. Isolated RNA can be quantified by Nanodrop, Qubit or equivalent device for RNA quantification. For data analysis requiring normalization, it may be beneficial to set up duplicate reactions. Use one reaction to prepare nuclear and cytoplasmic RNA and the second reaction to prepare total RNA from the same sample material. For details on various normalization options, please refer to Appendix Section H on page 13.

RNA purified using the RNA Subcellular Isolation Kit is suitable for use in a variety of downstream applications including RT-qPCR, Northern blot and RNA-seq. Additional DNase treatment can be performed on the purified RNA using Active Motif's DNase I Treatment Kit (Catalog No. 25502).

Determine the amount of starting material necessary to isolate sufficient RNA yields for downstream analysis by taking into consideration that ribosomal RNA (rRNA) accounts for a majority (~80%) of the total RNA in a cell. If you plan to perform rRNA depletion or poly adenylation selection following RNA isolation, you may need to increase the amount of starting material. Some general guidelines of RNA yields are provided below for reference.

Cell Number	Cytoplasmic RNA Yield	Nuclear RNA Yield	Total RNA Yield
2 Million	20 µg	20 µg	40 µg
1 Million	10 µg	10 µg	30 µg
500,000	5 µg	5 µg	15 µg
250,000	3 µg	3 µg	8 µg
100,000	1.5 µg	1.5 µg	3 µg

Section A: Preparation of Cultured Cells

The protocol below describes the preparation of one 150 mm plate. Each 150 mm plate should generate enough cells to prepare multiple RNA isolation reactions.

1. Grow cells to 90% confluence in a 150 mm plate. Treat cells if desired.
2. Wash cells once with 10 ml 1X PBS
3. Add 4 ml of Trypsin to each plate. Incubate at 37°C for 5 minutes.
4. Add 5 ml of complete media to each plate and pipet up and down to detach the cells from the plate. Transfer cells to a 15 ml conical tube.
5. Centrifuge for 5 minutes at 450 x g.

6. Remove the supernatant and resuspend the cells in 10 ml 1X PBS.
7. Count the cells using a hemacytometer and Trypan blue.
8. Set up reactions by adding desired number of cells to a microcentrifuge tube. If both subcellular fractionated and total RNA are needed, prepare two tubes for each sample. See table on page 7 for guidelines of RNA yields to determine the appropriate number of cells needed for downstream analysis. Discard any remaining cells.
9. Centrifuge the culture cell samples at 14,000 rpm for 5 minutes. Discard the supernatant.
10. Proceed to Section D for Nuclear & Cytoplasmic RNA Isolation. Proceed to Section E for Total RNA Isolation.

Section B: Preparation of Fresh Tissue

1. Add ice-cold 1X PBS to a conical of the appropriate size for the tissue of interest until the conical tube is 3/4 filled with PBS.
2. Place conical tube with PBS on a balance and tare the value to zero.
3. Working quickly, isolate your tissue of interest. Immediately wash with 10 ml ice-cold 1X PBS and add tissue to the tared conical tube. Record the mass of the tissue. Keep the tissue in PBS until ready to use. If preparing multiple samples, place tube on ice until all samples are ready.
4. We recommend working with 5 -15 mg of tissue per RNA isolation in microcentrifuge tubes. Based on the total mass of the initial tissue, prepare the appropriate number of microcentrifuge tubes. Add 1 ml ice-cold 1X PBS to each microcentrifuge tube.

Note: Any unused tissue can be transferred in 5-15 mg quantities to a cryo vial (without PBS). Flash freeze the cryo vial in liquid nitrogen and immediately store at -80°C for future use.

5. Transfer the tissue in PBS to an empty 150 mm cell culture plate. Use a sterile razor blade to dice the tissue into small pieces (~2 mm cubes).
6. Place a single microcentrifuge tube with 1 ml PBS on a balance and tare the value to zero. Transfer a small tissue section into the microcentrifuge containing the PBS. Continue until the desired mass is reached. Record the mass of the tissue. Place the tube back on ice.
7. Repeat step 6 until all of the tissue has been transferred to microcentrifuge tubes.
8. Keep the tissue samples to be processed on ice.
9. Shear the fresh tissue samples using a syringe and a large gauge needle (15-17). Continue to shear until the tissue is broken into smaller pieces.
10. Repeat shearing using a syringe and a medium gauge needle (21-23). Continue to break down the tissue into smaller pieces.
11. Repeat shearing using a syringe and a small gauge needle (27-29). The tissue should be a homogenous solution.

12. Place the sample on ice.
13. Repeat Steps 9-12 for each additional tissue sample until all samples have been processed.
14. Centrifuge the fresh tissue samples at 14,000 rpm for 5 minutes. Discard the supernatant.
15. Proceed to Section D for Nuclear & Cytoplasmic RNA Isolation. Proceed to Section E for Total RNA Isolation.

Section C: Preparation of Frozen Tissue

1. Use frozen tissue that has been flash frozen immediately after excision and stored at -80°C for no more than one month.
2. Chill a clean mortar and pestle that is free from RNase contamination.
3. Remove your frozen tissue sample from -80°C and place on dry ice.
4. We recommend working with 5 -15 mg of tissue per RNA isolation. If necessary, transfer the tissue to an empty 150 mm cell culture plate on dry ice. Working quickly, use a razor blade to dice the tissue into small pieces (~2 mm cubes). Do not allow the tissue to thaw. Any unused tissue should be stored at -80°C.
5. Transfer the desired amount of tissue to the chilled mortar and pestle and cover with liquid nitrogen. Use the pestle to grind the tissue into a fine powder. Collect the ground tissue into the center of the mortar using a sterile pipette tip.

Note: If preparing multiple reactions from the same tissue sample, the tissue can be prepared in bulk and then transferred to individual tubes.

6. Add 350 µl Complete Buffer G per reaction to the mortar to dissolve the tissue powder.

Note: For multiple reactions, scale up the amount of Complete Buffer G needed (*e.g.* Two reactions with 30 mg of tissue would use 700 µl Complete Buffer G).

7. Allow the mortar to warm up enough to transfer the dissolved tissue into a microcentrifuge tube. Add 350 µl per tube if working with multiple reactions. Place the samples in an ice bucket with ice until all samples have been processed.
8. Repeat steps 2-7 if working with multiple tissue samples, cleaning the mortar and pestle in between each sample.
9. Proceed to Section E, Step 2 to continue with total RNA isolation.

Section D: Nuclear & Cytoplasmic RNA Isolation

1. Resuspend each cell pellet in 120 µl Complete Lysis Buffer.
2. Incubate samples on ice: **Cultured cells:** Incubate for 10 minutes on ice.
Tissue samples: Incubate for 20 minutes on ice.
3. Centrifuge at 14,000 rpm for 5 minutes at 4°C.

4. Prepare a new RNase-free microcentrifuge tube for each sample and label as **Cytoplasmic**. Transfer the supernatant from Step 3 to the new tube. Take care to remove all of the supernatant without disturbing the pellet.
5. The remaining pellet contains the nuclear fraction. Label the tubes as **Nuclear**. Wash the nuclear pellets by adding 150 μ l of 70% ethanol to each tube. Centrifuge at 14,000 rpm for 5 minutes at 4°C. Carefully remove and discard supernatant.
6. Add Complete Buffer G to each tube as follows:
 - Cytoplasmic:** Add 250 μ l Complete Buffer G
 - Nuclear:** Add 350 μ l Complete Buffer G.
7. Vortex all tubes on highest setting for 30 seconds.
8. Add 350 μ l of 70% ethanol to all tubes.
9. Using a pipette and P200 tip, pipette up and down to homogenize the solution. (The solution is very gelatinous at first, but this should reduce with pipetting.)
10. Prepare separate purification columns for each cytoplasmic and nuclear sample. Add the entire sample to its corresponding purification column.
11. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
12. Add 600 μ l Wash Buffer to each column.
13. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
14. Add 600 μ l of 70% ethanol to each column.
15. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
16. Repeat centrifugation at 14,000 rpm for 2 minute at 4°C to remove any remaining liquid.
17. Transfer each column to a new RNase-free microcentrifuge tube. Make sure the new collection tubes are labeled for each sample.
18. Add 50 μ l DEPC water to the center of each column. Let stand for 1 minute.
19. Centrifuge the columns at 14,000 rpm for 1 minute at 4°C. Eluate contains the Subcellular RNA fractions.
20. Discard the columns. Place the nuclear and cytoplasmic RNA samples on ice.
21. Proceed to Section F for RNA quantification.

Section E: Total RNA Isolation

1. Resuspend each cell pellet in 350 μ l Complete Buffer G.
2. Vortex tubes on highest setting for 30 seconds.

3. Add 350 μ l of 70% ethanol to each tube.
4. Using a pipette and P200 tip, pipette up and down to homogenize the solution. (The solution is very gelatinous at first, but this should reduce with pipetting).
5. Prepare separate purification columns for each sample. Add the entire sample to its corresponding purification column.
6. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
7. Add 600 μ l Wash Buffer to each column.
8. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
9. Add 600 μ l of 70% ethanol to each column.
10. Centrifuge the columns with their collection tubes at 14,000 rpm for 1 minute at 4°C. Discard the flow through.
11. Repeat centrifugation at 14,000 rpm for 2 minute at 4°C to remove any remaining liquid.
12. Transfer each column to a new RNase-free microcentrifuge tube. Make sure the new collection tubes are labeled for each sample.
13. Add 50 μ l DEPC water to the center of each column. Let stand for 1 minute.
14. Centrifuge the columns at 14,000 rpm for 1 minute at 4°C. Eluate contains the total RNA.
15. Discard the columns. Place total RNA samples on ice.
16. Proceed to Section F for RNA quantification.

Section F: RNA Quantification

1. Quantify RNA using the absorbance 260nm value with a Nanodrop or equivalent spectrophotometric device. If anticipating low RNA yield, use a fluorescent RNA detection method, such as Qubit, to quantify the RNA samples. For accurate quantification it is important that the sample measurement falls within the detectable range of the instrument used.
2. We recommend preparing aliquots of the RNA and storing at -20°C to -80°C. It is important to avoid freeze/thaw cycles when working with RNA.

Appendix

Section G. Downstream Applications

Purified RNA is suitable for use in a variety of downstream applications, including reverse transcription PCR (RT-qPCR), Northern blotting and RNA-seq.

DNase I Treatment

If desired, RNA samples can be treated with DNase I to ensure the removal of genomic DNA from the nuclear or total RNA fractions. It is recommended to use Active Motif's DNase I Treatment Kit (Catalog No. 25502) or equivalent DNase treatment protocol. Following DNase treatment, the RNA sample should be quantified using the methods described in Section F.

RT-qPCR

For RT-qPCR we recommend the use of a two-step real time PCR in which the reverse transcription reaction is performed separately from the real-time PCR assay. Commercially available reverse transcription kits (e.g. iScript™ cDNA Synthesis Kit, Bio-Rad Cat No. 1708890) can be used to generate cDNA. Follow the manufacturer's recommendations for reverse transcription and qPCR. Section H below discusses considerations for control reactions and data normalization.

RNA-Seq

For RNA-seq, we recommend checking with your sequencing facility to determine their requirements for RNA material. Many sequencing facilities will perform poly adenylation selection or rRNA depletion as part of the library preparation process. The sequencing facility will also provide guidelines on the amount of RNA required.

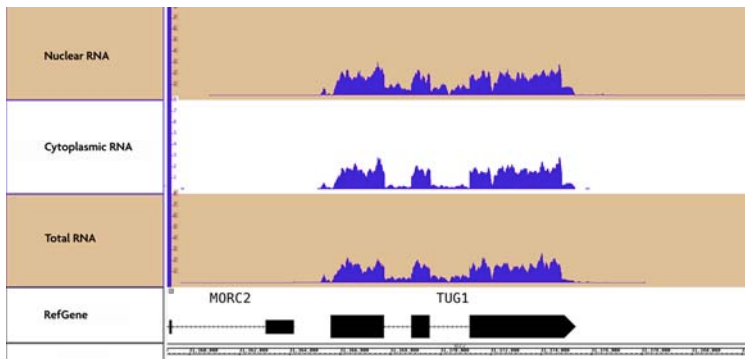


Figure 4: RNA-Seq data using RNA fractions isolated with the RNA Subcellular Isolation Kit.

Nuclear, cytoplasmic and total RNA were isolated from HeLa cells using the RNA Subcellular Isolation Kit. RNA was subjected to ribosomal RNA depletion during NGS library preparation. Samples were then sequenced using the Illumina HiSeq and 100 bp paired end reads with 50 M reads per sample. TUG1 is a lncRNA known to have both nuclear and cytoplasmic localizations.

Section H. Data Normalization

Gene expression analysis using real time reverse transcription quantitative PCR (RT-qPCR) is often the method of choice for accurate expression profiling of select genes. This method is highly sensitive, reproducible and has a large dynamic range. However, it is important to consider proper controls for gene normalization to account for differences in starting material, RT efficiency, RNA integrity and RNA localization (if evaluating different subcellular fractions). We will present two approaches for data normalization below. Determine the appropriate method for your research analysis.

Normalization using a reference gene

Data normalization can be performed by including an endogenous control in the assay to correct for sample-to-sample variations. This control is usually a reference gene that is constitutively expressed (also referred to as a housekeeping gene). Individual RT-qPCR samples are normalized to the reference gene as a fold-change to allow for direct comparison between different samples. The challenge with this approach is to select the appropriate control gene. The gene should not show variation in expression in the tissues or cells being investigated, nor should it change in response to experimental treatments. Another consideration when evaluating RNA samples from different subcellular fractions is the localization of the control gene. If the control gene is localized to one subcellular fraction, use of this gene for analysis with a different subcellular fraction can bias the results. Therefore, it is often necessary to use several reference genes.

$$\text{Fold change} = 2^{\text{-(Ct control gene - Ct test gene)}}$$

Normalization using total RNA content

Data normalization is also performed against total cellular RNA. This method requires accurate quantification of the total RNA fraction. RNA extracted from nuclear and cytoplasmic fractions can be compared to the total RNA levels. By using total RNA for normalization, bias that is the result of RNA localization is avoided. However, it does require the preparation of nuclear, cytoplasmic and total RNA from the same sample material.

$$\% \text{ of Input} = 100 \times [2^{\text{-(Ct total RNA - Ct RNA fraction)}}]$$

Section K: Troubleshooting Guide

Problem/question	Recommendation
Little or no recovery of RNA	Make sure to use RNase-free tubes, tips and reagents when working with RNA samples. Wear gloves at all times when handling the samples.
	Quantify RNA using a spectrophotometer or fluorescent detection method. For accurate quantification it is important that the sample measurement falls within the detectable range of the instrument used.
	Quantification of RNA may be influenced by the presence of genomic DNA in the sample. If desired, perform a DNase treatment of the isolated RNA. Expect to see a reduction in the yield following DNase treatment.
I see genomic DNA in my sample	The RNA Subcellular Isolation Kit can be used to isolate RNA from nuclear and cytoplasmic fractions and total RNA. It is normal to see genomic DNA isolated in the nuclear and total RNA samples. To remove genomic DNA from the RNA samples, we recommend using Active Motif's DNase I Treatment Kit (Catalog No. 25502).
Is it normal for my sample consistency to change after addition of 70% ethanol?	Yes, the combination of the Complete Buffer G and the 70% ethanol will change the consistency of the sample. Use a P200 pipette and tip and mix the sample by pipetting up and down 15-20 times. The sample consistency should become easier to work with after pipetting. If the sample continues to be difficult to work with, use a 27-29 gauge needle and syringe to break up the material.
Can I elute in less volume?	We recommend elution from the purification columns with 50 µl DEPC water. Eluting in smaller volumes significantly reduces the amount of RNA recovered. If necessary, RNA can be concentrated with additional purification steps following elution off the column.
What species can I use this kit for?	The RNA Subcellular Isolation Kit has been used to enrich for RNA from mammalian samples. We have not tested with other species.
Storage of RNA	RNA samples may be stored at -20°C to -80°C. We recommend preparing smaller aliquots of RNA to avoid freeze/thaw cycles.

Section L: Related Products

LncRNA Promoters	Format	Catalog No.
LightSwitch™ FIRRE Promoter Reporter Vector	5 µg	32071
LightSwitch™ GASS Promoter Reporter Vector	5 µg	32072
LightSwitch™ HOTAIR Promoter Reporter Vector	5 µg	32073
LightSwitch™ MALAT1 Promoter Reporter Vector	5 µg	32074
LightSwitch™ MEG3 Promoter Reporter Vector	5 µg	32075
LightSwitch™ TUG1 Promoter Reporter Vector	5 µg	32076

miRNA Target Validation	Format	Catalog No.
miRNA Target IP Kit	10 rxns	25500

Luciferase Assay	Format	Catalog No.
LightSwitch™ Luciferase Assay Kit	100 assays	32031
	1000 assays	32032

Active Motif offers a large collection of promoter and 3'UTR elements that have been cloned into LightSwitch luciferase reporter constructs. These reporters are transfection ready and work with the LightSwitch Luciferase Assay System. The 3'UTRs can be used in combination with miRNA mimics and inhibitors. For a complete list go to www.activemotif.com/lightswitch

Active Motif also offers a growing list of application validated antibodies, including antibodies for histones and histone modifications, transcription factor antibodies, DNA methylation-related antibodies and CHIP validated antibodies. For a complete list go to www.activemotif.com/abs

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008, USA
E-mail: tech_service@activemotif.com

Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351

Active Motif Europe

Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
E-mail: eurotech@activemotif.com
Direct: +32 (0)2 653 0001

Germany Free Phone: 0800 181 99 10
France Free Phone: 0800 90 99 79
UK Free Phone: 0800 169 31 47
Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan

Direct: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

787 Kangqiao Road
Building 10, Suite 202
Pudong District
Shanghai, 201315, China

Direct: (86)-21-20926090
Hotline: 400-018-8123
E-mail: techchina@activemotif.com

Visit Active Motif on the worldwide web at <http://www.activemotif.com>

At this site:

- Read about who we are, where we are, and what we do
- Review data supporting our products and the latest updates
- Enter your name into our mailing list to receive our catalog, *MotifVations* newsletter and notification of our upcoming products
- Share your ideas and results with us
- View our job opportunities

Don't forget to bookmark our site for easy reference!