Datasheet

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ExoRNA Extraction kit

This product is for research use only.
It is highly recommended to read this users guide in its entirety prior to using this product.
Do not use this kit or its components beyond the indicated expiration date.

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TABLE OF CONTENTS

PRODUCT DESCRIPTION

Product overview

Exosomes shuttle functional RNA molecules in the target cell. Increasing evidence suggests a role for exosome-derived miRNAs in the development and/or progression of specific human diseases. Pathogenic miRNAs might be exploited as novel therapeutic targets or disease biomarkers in complex diseases, including cancer. In fact, miRNAs seem to play critical roles as transcriptional and post-transcriptional regulators of epigenetic mechanisms and cell processes and have been linked to the etiology, progression and prognosis of cancer. Similar miRNA expression patterns between tumor tissue samples and circulating exosomes have been observed.

AMSBIO has developed optimized solutions for the efficient extraction of high-quality totalRNA (miRNA and mRNAs) from the overall exosomes and microvesicle population or from tumor-specific exosome subpopulation, which helps to facilitate the identification of tumor miRNA or mRNAs signatures from human biofluids or cell culture media.

PRODUCT CONTENT

Description	Component	Amount
Immunobeads	Pre-coupled latex Immunobeads 20 or 40 reactions	1 vial (20 or 40 reactions)
Exosome Standards for positive control	Depend on the sample type used, customer may choose between human plasma, serum, urine or cell culture supernatant exosomes.	1 vial (100 µg)
Beads Washing buffer 5X	Buffer for washing beads	1 bottle (10 ml, 20 reactions) 1 bottle (20 ml, 40 reactions)
RNA Washing buffer	Buffer for washing columns (to add Ethanol 96%)	1 bottle (9 ml, 20 reactions) 2 bottles (9 ml, 40 reactions)
Lysis buffer	Solution for exosome lysis	1 bottle (16 ml, 20 reactions) 1 bottle (30 ml, 40 reactions)
Elution buffer	Buffer for column elution	1 vial (1ml)
Columns	Columns for RNA extraction (assembled with one tube)	22 columns/20 reactions 42 columns/40 reactions
Elution tubes	RNase free microfuge tubes (1.5 ml) for RNA Elution	22 tubes/20 reactions 42 tubes/40 reactions

Other material required

- Single-use and/or pipettes with disposable tips 2-100 µl
- Pipettes 1 ml and 5 ml for reagent preparation
- PBS
- Disposable pipetting reservoirs
- Ethanol 96%
- Chloroform or BCP (1brome-3chlorepropane)
- Sample concentrator (urine and cell culture supernatant samples)

STORAGE INFORMATION

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- All reagents, immunobeads and buffers provided within the RNA Extraction Kit must be stored at 4°C.
- Spin columns and Elution tubes must be stored at room temperature
- DO NOT FREEZE!

PROCEDURE FOR RNA EXTRACTION

STEP A: Sample preparation:

1. Prepare **plasma and serum samples** by 3 centrifugation steps to eliminate red blood cells and cellular debris.
   - 10′ at 300 g (save supernatant; discard pellet)
   - 20′ at 1 200 g (save supernatant; discard pellet)
   - 30′ at 10 000 g (save supernatant; discard pellet)

2. Preclear **urine samples** by following this protocol:
   - Centrifuge at 16 000 g for 20′ at room temperature.
   - Filter by using 0.45 µm filter.
   - Concentrate urine samples by spin concentrator 15-20 times.*

3. Prepare **cell supernatant samples** by following this protocol:
   - 10′ at 300 g (save supernatant; discard pellet)
   - 20′ at 1 600 g (save supernatant; discard pellet)
   - 30′ at 10 000 g (save supernatant; discard pellet)
   - Concentrate cell supernatant 10-20 fold in spin concentrator.*

*The quantity of exosomes could vary between samples. Concentration factors are given for information purposes only, a larger starting amount of sample should be used if the signal is weak.

STEP B: Reagent preparation

1. **Beads Washing Buffer**
   - Ensure there is no crystal precipitate.
     NOTE: If crystals are observed, dissolve them by warming up the concentrated 5x Washing Buffer bottle at 37°C before proceeding with the dilution.
   - Dilute with deionized water Bead Washing Buffer 5X to 1X.

2. **RNA Washing Buffer**
   - Add into the bottle containing RNA Washing Buffer the volume of pure ethanol (96%) indicated on the bottle's label to get the final ethanol concentration of approximately 70%.

3. **Elution buffer and Lysis buffer are ready to use.**

STEP C: Exosomes binding

- **PURIFIED EXOSOMES (Lyophilized Standards)**
  - Purified exosomes do not require this binding step. If the samples are purified exosomes, skip to STEP D: Extraction method.

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- **UNFRACTIONATED SAMPLES**
  - Place 0.1 ml up to 1 ml of sample into low-binding tubes (not provided in the kit) - Volumes suggested: 0.1 ml up to 0.5 ml for small RNAs analysis; 0.5 ml up to 1 ml for mRNAs analysis.
  - Add PBS 1X to the sample to get a final volume of 1 ml. (If you are using 1 ml of plasma dilution is not necessary).
  - Add 10 µl of Immunobeads.
  - Incubate sample-Immunobead mixture overnight at 4°C in rotator.
  - Centrifuge at room temperature (RT) for 10' at 5 000g
  - Discard supernatant.
  - Wash beads:
    - Add 1 ml of Beads Washing Buffer.
    - Resuspend up and down 10-15 times.
    - Centrifuge at RT for 10' at 5 000g.
    - Remove the supernatant being careful not to disturb the pellet.
    - Wash beads once again as indicated above.

STEP D: RNA Extraction

- **LYSIS**
  - **PURIFIED EXOSOMES**
    - Add 200 µl of Lysis buffer directly onto lyophilized exosomes.
    - Resuspend by pipetting and transfer to a fresh tube.
    - Add 500 µl of Lysis buffer to reach a final volume of 700 µl.
    - Incubate 5' at room temperature.

  - **UNFRACTIONATED SAMPLES**
    - Add 700 µl of Lysis buffer directly on the bead pellet.
    - Dissolve the pellet by pipeting up and down (beads must be totally dissolved).
    - Incubate 5' at room temperature.

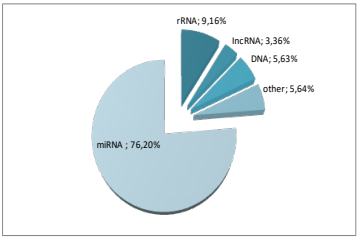
- **EXTRACTION**
  - Add 70 µl of 1-Brome-3-chlorepropane (BCP) or 140 µl of pure Chloroform.
  - Shake 30 seconds.
  - Incubate 10 minutes at room temperature.
  - Incubate 1 minute in ice and centrifuge at 12 000g at 4°C for 10'.
  - NOTE: Incubation on ice prior to centrifuge helps reducing DNA contamination, which tend to remain in the interphase.
  - Transfer the top phase (aqueous) to a fresh tube.
  - Add 2X of ethanol 96%. Mix by gently inverting 4 - 5 times.
    - if the top phase volume is 400 µl add 800 µl of ethanol 96 %.

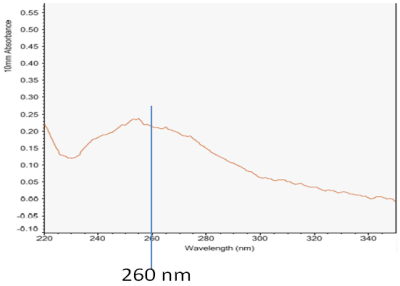
- **PURIFICATION**
  - Transfer the half volume of the mixture into spin column.
  - Spin at 14 000 g for 30".
  - Discard the flow-throw.
  - Add the remaining volume into the same spin column.
  - Spin at 14 000 g for 30".
  - Discard the flow-throw.
  - Wash column with RNA Washing buffer.
    - Add in the column 400 µl of RNA Washing buffer.
    - Gently invert the column 3 - 4 times
    - Spin at 14 000g for 30".
    - Discard flow-through.
    - Perform the washing step twice more.
  - Spin 5 additional minutes at 14 000g to eliminate ethanol residues from column
  - Remove the tube and transfer the spin column into an elution tube.
  - Elute the column with 15 µl of Elution buffer.
  - Incubate 5' at Room Temperature.
  - Spin 2' at 200 g and 1' at 14 000 g. Keep flow-through.
  - Eluted RNA is now ready for downstream analysis or for storage at -80°C.

DATA ANALYSIS

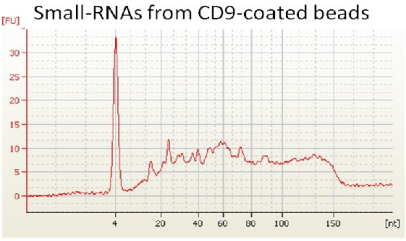
Nanodrop analysis

Purified exosome RNA can be quantified and analyzed using NanoDrop spectrophotometer (Thermo Scientific), although the measured concentration values are likely to end toward the bottom limit of detection of the instrument. For better quantification, we recommend the concomitant use of electropherogram-based technologies (eg Bioanalyzer, Agilent Technologies) or fluorimetric technologies (Qubit nano; Themro Scientific). Since most of the RNA contained in extracellular vesicles are small-non-coding RNAs (eg. miRNA), the expected Nanodrop profile, purity and yield are as shown in the representative figures 1 (for Nanodrop profiles and RNA contamination see troubleshooting guide)





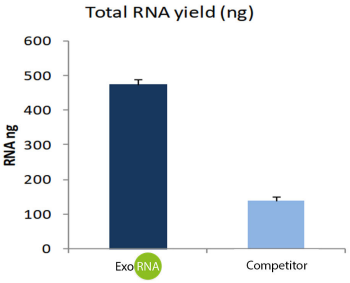
1. Expected Nanodrop profile for RNA extracted from immunocaptured exosomes (100 µl of human plasma).
Yield = 8,4 ng/µl; A260/280 =1,6; A260/230= 1,85 .



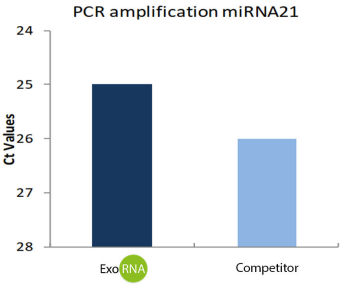
2. RNA quality control with Agilent Bioanalyzer.

EXO-Total RNA allows extraction of high quality of exosome-derived RNAs from low volumes of sample

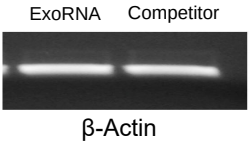
Extraction of total exosome RNA was performed from 100 µl of healthy donor plasma (HD #1 an #2) with Competitor Kit or with the ExoRNA Kit (AMSBIO). RNA yield was quantified by Nanodrop (Fig 3) and extracted RNA was subsequently retrotranscribed using the miScript II RT kit (Qiagen). miR-21 and b-actin markers were amplified by qPCR (Fig 4 and 5).



3. Nanodrop quantification of total RNA yield

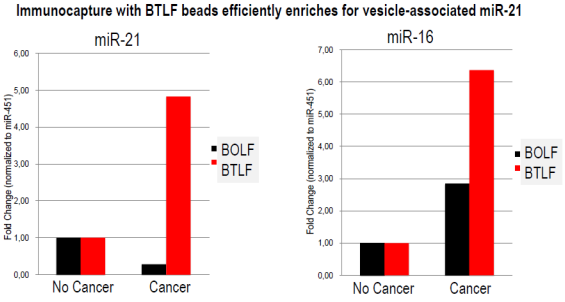


4. miRNA 21 amplification by qPCR



5. β-Actin amplification by PCR

Enrichment and analysis of cancer-derived miRNA from plasma EVs



Total EV derived RNA was extracted from 100 µl of plasma from a healthy donor and a cancer patient using ExoRNA containing beads for overall (BOLF) or tumor derived (BTLF) exosome capture, respectively. Total RNA extracted was retrotranscribed using the miScript II RT kit (Qiagen) and the expression level of miR-21 and miR-16 were measured relative to control miR451 by qPCR

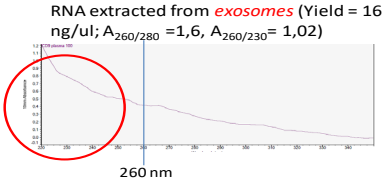
TROUBLESHOOTING

General notes and safety recommendations on handling RNA

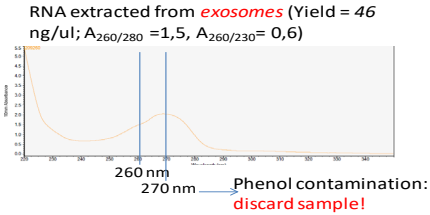
- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice when aliquots are pipetted for downstream applications.
- Reduce the preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure. (These tubes are generally RNase-free.)
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware have to stand for 12 hours at 37°C and then autoclave or heat to 100°C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- All buffers must be prepared from DEPC-treated RNase-free ddH2O.
- Do not use equipment, glassware and plasticware employed for other applications which might introduce RNase contaminations in the RNA isolation.

Nanodrop Profiles and RNA contaminations

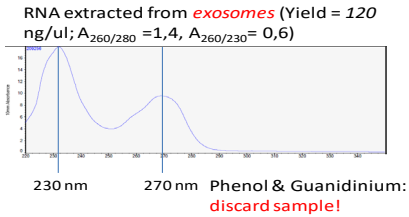
Low guanidinium hydrochloride contaminations can be present without affecting the RNA quantification (fig 6). Nanodrop profiles that show a peak at 270 nm (fig 7) or 2 picks at 230 nm and 270 nm (fig 8) respectively, are index of phenol or phenol/guanidimium contaminations, that affect the estimate of RNA yield, and downstream analyses. In these cases (fig 7 and 8) we suggest to discard the samples and repeat the RNA extraction.



6. Guanidinium contamination does not affect RNA quantitation neither downstream analysis



7. Phenol contamination causes an over-extimation of RNA yield and affects downstream analyses.



8. Phenol and guanidinium contamination. Discard the sample and repeat the RNA extraction

Problem/ Possible Cause	Suggested Solution
Degraded RNA	
Degraded RNA	RNA is very sensitive to degradation by endogenous and exogenous RNases in the biological material used for RNA extraction. For the isolation of undegraded total RNA, it is vital to use the freshest biological material available. Even storage of tissue, cells or blood at -80°C causes RNA degradation with time, as well as during the thawing process. Whenever possible, the RNA isolation should be carried out immediately after the collection of the biological material. If the biological material is to be stored or shipped to another laboratory before the RNA purification can take place, the samples should be stored under the Lysis Solution. The chaotropic compounds in the Lysis Solution inhibit endogenous RNases thus preventing RNA degradation in the sample even at ambient temperature.
Clogged Spin Filter	
Insufficient disruption or homogenization of starting material	After lysis spin lysate to pellet debris and continue with the protocol using the supernatant. Increase g-force and/ or centrifugation time. Reduce amount of starting material.
Little or no total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer to 5-10 min or repeat elution step once again.
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is frozen immediately in liquid N_2 and is stored continuously at – 80°C! Avoid thawing of the material. Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contaminations of solutions, receiver tubes etc.	Use sterile, RNase-free filter-tips. Before every preparation clean up the pipettes, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications	
Ethanol carryover during elution	Increase g-force or centrifugation time.
Salt carryover during elution	Ensure that Washing Buffer are at room temperature. Check up Washing Buffer for salt precipitates. If there are any precipitates solve these precipitates by careful warming.

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