PRODUCT INFORMATION & MANUAL

Exosome RNA (Pre-Isolated Exosomes) Extraction Kit

NBP3-14801

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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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.

Optimized solutions have been developed for the efficient extraction of high-quality total RNA (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.

About Exosomes

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

RNA extraction kit:

Exosome RNA (Pre-Isolated Exosomes) Extraction Kit (NBP3-14801):

Kit allows RNA extraction from exosomes pre-isolated with different methods (ultracentrifugation, chemical precipitation, immunocapture, size-chromatography etc).

PRODUCT CONTENT

Description	Component	Amount
RNA Washing buffer	Buffer for washing columns (to add Ethanol 96%)	1 bottle (10 ml, 25 reactions)
Lysis buffer	Solution for exosome lysis	1 bottle (19 ml, 25 reactions)
Elution buffer	Buffer for column elution	1 vial (1ml)
Columns	Columns for RNA extraction (assembled with one tube)	27 columns/25 reactions
Elution tubes	RNase free microfuge tubes (1.5mL) for Elution	27 tubes/25 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: Reagent preparation

- **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%.

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

STEP B: RNA Extraction from pre-isolated exosomes

- **LYSIS**
  - Add 700 µl of Lysis buffer directly onto the exosome preparation.
  - Resuspend by pipeting up and down until the lysate is clear.
  - 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 %.

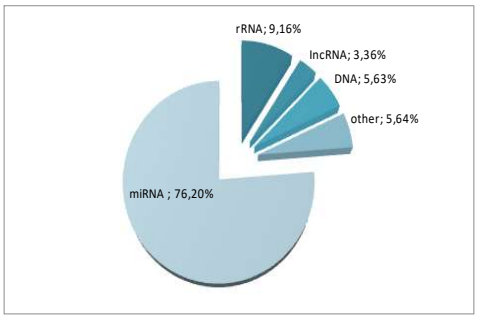
- **PURIFICATION**
  - Transfer the half volume of the mixture into spin column.
  - Spin at 14 000 g for 30".
  - Discard the flow-throw.
  - Add the remaining volume into the same spin column.
  - Spin at 14 000 g for 30".
  - Discard the flow-throw.

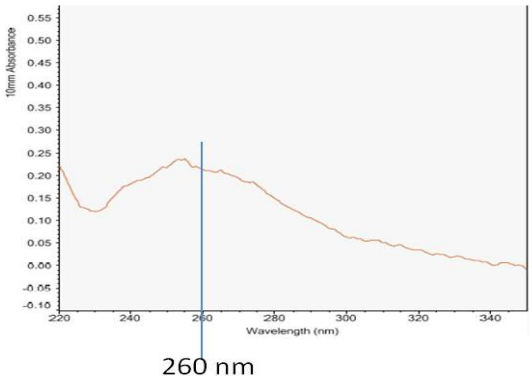
- Wash column with RNA Washing buffer.
    - Add in the column 400 µl of RNA Washing buffer.
    - Gently invert the column 3 - 4 times
    - Spin at 14 000g for 30''.
    - Discard flow-through.
    - Perform the washing step twice more.
- Spin 5 additional minutes at 14 000g to eliminate ethanol residues 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

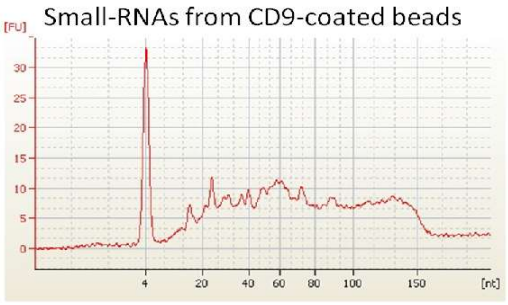
Nanodrop analysis

Purified exosome RNA can be quantified and analyzed using NanoDrop spectrophotometer, 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 or fluorimetric technologies. 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)



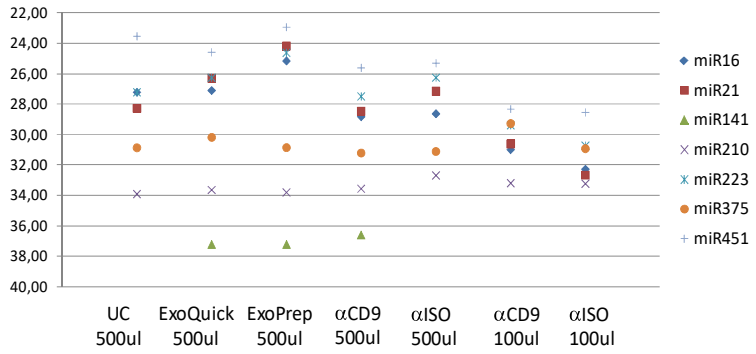


1. Expected Nanodrop profile for RNA extracted from immunocaptured exosomes (100 µl of human plasma).
Yield = 8,4 ng/µl; A260/280 =1,6; A260/230= 1,85 .



2. RNA quality control with Agilent Bioanalyzer.

Isolated RNA is suitable for downstream analyses, as miRNA profiling



Legend: UC-Ultracentrifuged; ExoQuick- Isolated with ExoQuick reagent; EXOPrep-Isolated with EXO-Prep, αCD9- Isolated with CD9-coated beads; αISO Isolation with mouse isotype antibody coated beads (neg control for immunoaffinity capture)

Expression profile of seven circulating miRNA were compared between different isolation methods from human plasma. RNA was extracted with Kit and retro-transcribed with miScript II RT kit.

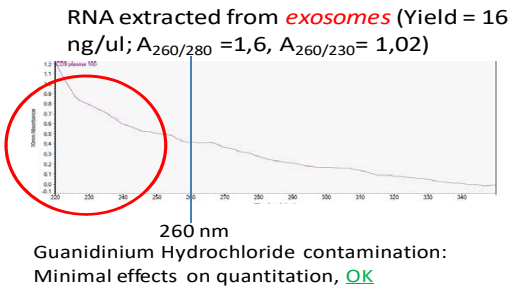
TROUBLESHOOTING

General notes and safety recommendations on handling RNA

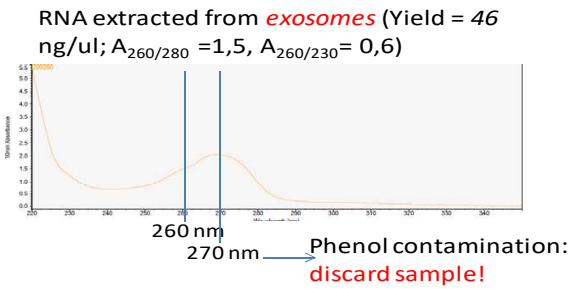
- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice when aliquots are pipetted for downstream applications.
- 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

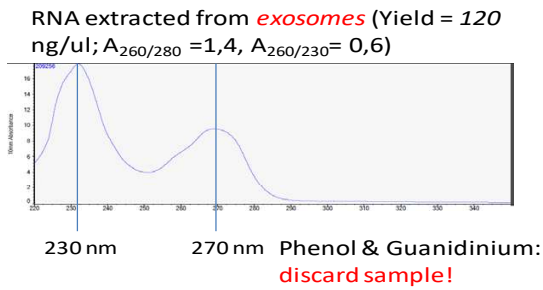
Low guanidinium hydrochloride contaminations can be present without affecting the RNA quantification (fig 6). Nanodrop profiles that show a peak at 270 nm (fig 7) or 2 picks at 230 nm and 270 nm (fig 8) respectively, are index of phenol or phenol/guanidimium contaminations, that affect the estimate of 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-estimation 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.