Basic RNA Extraction Kit (BN00082)

(Cat#: BN00082 -25, -50; Store at +4°C)

I. Introduction:

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 the amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases. 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.

Basic RNA extraction kit allows total RNA (miRNA + mRNAs) extraction from pre-isolated exosomes pre-isolated via different methods including ultracentrifugation, chemical precipitation, immunocapture, size-chromatography etc). *RNA basic kit does not contain immunobeads for exosome isolation. Pre-isolated exosomes are lysed with an optimized lysis buffer and total RNA is purified using spin columns with a fast and user-friendly process. Eluted RNA can be used for downstream analyses or stored at -80°C. Exosome standards for positive control are also included in the kit. All our kits guarantee high specificity for exosomal RNA and high yield of total RNA (including small RNAs) than similar products.

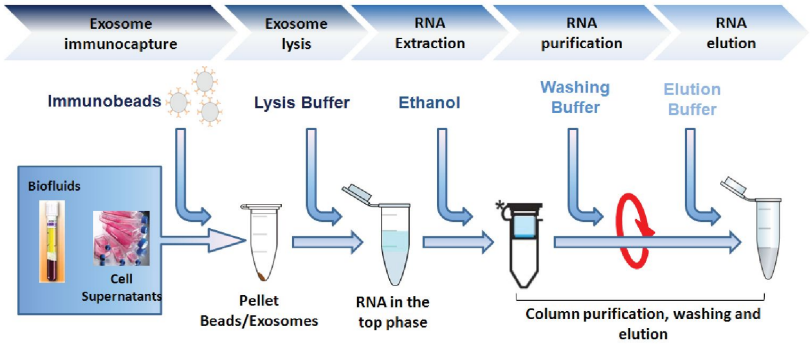

Figure 1. Exosomes RNA Extraction Kit.

II. Application:

- **Exosome RNA extraction from pre-isolated exosomes pre-isolated via different methods including ultracentrifugation, chemical precipitation, immunocapture, size-chromatography etc**
- Simultaneous miRNA and mRNA profiling (qRT-PCR, RT-PCR, microarray).

III. Sample Type:

- Pre-isolated exosomes from different samples (biofluids or cell culture media, either pellet after precipitation, frozen, or lyophilized).

IV. Kit Contents (for RNA extraction from Plasma, Serum, Urine, Cell culture media, pre-isolated exosomes):

Components	BN00082 25 reactions	BN00082 50 reactions	Part Number	Storage Temperature
Lysis buffer	1 bottle (19 ml)	1 bottle (37 ml)	BN00082-1	+4°C
RNA Washing buffer* (to add Ethanol 96%)	1 bottle (10 ml)	2 bottles (10 ml)	BN00082-2	+4°C
	1 vial (1 ml)	1 vial (1 ml)	BN00082-3	+4°C
Elution buffer	27 columns	52 columns	BN00082-4	+4°C or RT
Columns	27 tubes	52 tubes	BN00082-5	+4°C or RT
Elution tubes				

***Add 24 ml of Ethanol 96% to the RNA Washing Buffer to BN00082-25. *Add 48 ml of Ethanol 96% to the RNA Washing Buffer to BN00082-50.**

V. User Supplied Reagents and Equipment:

- 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 (1-bromo-3-chloropropane)
- Sample concentrator (urine and cell culture supernatant samples)

VI. Shipment and Storage:

All the reagents and buffers with the RNA extraction kit are shipped and stored at +4°C for up to 24 months, if unopened. Spin columns and Elution tubes may be stored at RT. DO NOT FREEZE!

VII. **Reagent Preparation and Storage Conditions:**

- RNA Washing Buffer. Add into the bottle containing RNA Washing Buffer the volume of pure ethanol (96%) mentioned above to get the final ethanol concentration of approximately 70% ethanol.
- Elution buffer and Lysis buffer are ready to use.
- The RNase free column and elution tubes should be stored at room temperature.
- All the opened buffers and diluted reagents including the bead washing buffer, RNA washing buffer, lysis buffer and the elution buffer should be stored at 4°C.

VIII. **Assay Protocol:**

1. **RNA Extraction:**

a) Lysis:

a. Add 700 µl of Lysis buffer directly onto the exosome preparation.
b. Resuspend by pipetting up and down until the lysate is clear.
c. Incubate for 5 min at room temperature.

b) **Extraction:**

a. Add 70 µl of 1-Bromo-3-chloropropane (BCP) or 140 µl of pure Chloroform.
b. Shake for 30 sec.
c. Incubate for 10 min at room temperature.
d. Incubate for 1 min on ice and centrifuge at 12,000g at 4°C for 10 min.
e. NOTE: Incubation on ice prior to centrifugation helps in reducing DNA contamination, which tend to remain in the interphase.
f. Transfer the top phase (aqueous) to a fresh tube.
g. 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%.

c) **Purification:**

a. Transfer the half volume of the mixture into the spin column.
b. Spin at 14,000g for 30 sec.
c. Discard the flow-throw.
d. Add the remaining volume into the same spin column.
e. Spin at 14,000g for 30 sec. Discard the flow-throw.
f. Wash the column with RNA Washing buffer. Add 400 µl of RNA Washing buffer in the column. Spin at 14,000g for 30 sec. Discard the flow-through. Perform the washing step twice more.
g. Spin for 5 additional min at 14,000g to eliminate any ethanol residues from the column. Discard the flow-throw.
h. Remove the tube and transfer the spin column into an elution tube.
i. Elute the column with 15 µl of Elution buffer. Incubate for 5 min at room temperature. Spin for 2 min at 200g and 1 min at 14,000g. Keep the flow-through. Eluted RNA is now ready for downstream analysis or for storage at -80°C

IX. **Sensitivity:** 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 fluorometric 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 Figure 2. This kit allows extraction of high quality of exosome-derived RNAs from low volumes of sample and better performance than competitors. Efficiency was tested vs a competitor kit for RNA extraction from healthy donor plasma derived exosomes. RNA yield was quantified by Nanodrop (Figure 3) and extracted RNA was subsequently retrotranscribed. miRNA21 was amplified by qPCR (Figure 4).

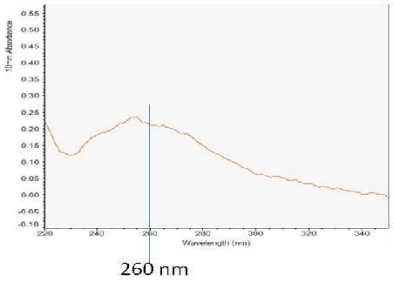

Figure 2. Expected Nanodrop profile for RNA extracted from immunocaptured exosomes (100 µl of human plasma). Yield = 8.4 ng/µl; $A_{260/280}$ =1.6; $A_{260/230}$= 1.85

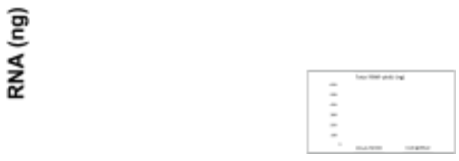

Figure 3. Nanodrop quantification of total RNA yield

Figure 4. miRNA 21 amplification by qPCR

X. General Troubleshooting Guide:

Problems	Cause	Solution
Degraded RNA	. Degraded RNA	. RNA is very sensitive to degradation by the 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 temperatures.
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 the g-force and/or the centrifugation time. . Reduce the amount of the starting material.
Little or no total RNA eluted	. Insufficient disruption or homogenization . Incomplete elution	. Reduce amount of starting material. Overloading reduces yield! . Prolong the incubation time with Elution Buffer to 5-10 min or repeat the elution step once again.
Total RNA degraded	. RNA source inappropriately handled or stored . RNase contaminations of solutions, receiver tubes etc	. Ensure that the starting material is frozen immediately in liquid N2 and is stored continuously at -80°C. Avoid thawing of the material. Ensure that the protocol, especially the first steps, has been performed quickly. . 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 . Salt carryover during elution	. Increase the g-force or the centrifugation time. . Ensure that Washing Buffer is at room temperature. Check the Washing Buffer for salt precipitates. If there are any precipitates solve these precipitates by careful warming.

FOR RESEARCH USE ONLY! Not to be used on humans.