Exosome Immunocapture and RNA Extraction Kit (BN00079)

(Cat# BN00079; 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 the 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.

This overall exosome immunocapture and RNA extraction kit has been developed for the isolation and efficient extraction of high-quality total RNA (miRNA and mRNAs) from the overall exosome population from human biofluids or cell culture media. Exosome capture occurs on immunobeads precoated with exosome associated antibodies enabling overall exosome isolation. The immunobeads can capture the overall exosome population from a wide range of media, including cell culture supernatants and human biofluids (plasma, serum, urine, etc) as well as enrich some specific exosome subpopulations (tumor-derived exosomes). The captured exosomes are subsequently 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.

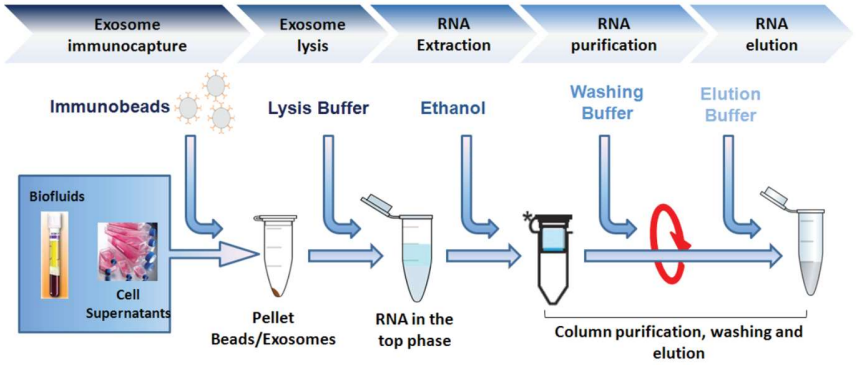

Figure 1. Exosome RNA Extraction Kit.

II. Application:

- Direct capture and exosome RNA extraction from human biofluids and cell culture media without initial exosome purification step.
- Starting from 0.1 ml of biological sample (plasma) per reaction. Whole plasma and serum can be directly used for capture. Concentrated urine and cell culture supernatant samples are recommended prior capture according to our suggested protocol.
- Simultaneous miRNA and mRNA profiling (qRT-PCR, RT-PCR, microarray).

III. Sample Type:

- Human biological fluids including Plasma, Serum, Urine and Cell culture media.

IV. Kit Contents (for Overall Exosome Immunocapture and RNA Extraction from human biofluids and cell culture media):

Components	BN00079	Part Number
	20 Assays	
Pre-couple latex immunobeads	1 vial (20 reactions)	BN00079-1
Bead Washing buffer (5X)	1 bottle (10 ml)	BN00079-2
Lysis buffer	1 bottle (16 ml)	BN00079-3
RNA Washing buffer (to add Ethanol 96%)	1 bottle (9 ml)	BN00079-4
Elution buffer	1 vial (1 ml)	BN00079-5
Columns	22 columns	BN00079-6
RNase free elution tubes (1.5 ml)	22 tubes	BN00079-7
Lyophilized Exosome Standard	1 vial (100 µg)	BN00079-8

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 are shipped and stored at 4°C for up to 8 months, if unopened. Briefly centrifuge small vials prior to opening. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- Immunobeads can be stored at +4°C for up to 8 months.
- The RNase free columns and elution tubes must 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. Exosome Immunocapture and RNA Extraction Kit Assay Protocol:

1. Plasma and Serum sample preparation: Prepare plasma samples by 3 centrifugation steps to eliminate red blood cells and cellular debris.

a) 10 min at 300g (save supernatant; discard pellet).
b) 20 min at 1200g (save supernatant; discard pellet).
c) 30 min at 10,000g (save supernatant; discard pellet).

2. Urine sample preparation:

a) Centrifuge at 16,000g for 20 min at room temperature.
b) Filter by using 0.45 µm filter.
c) Concentrate urine samples by spin concentrator 15-20 times*.

3. Cell supernatant sample preparation:

a) 10 min at 300g (save supernatant; discard pellet).
b) 20 min at 1600g (save supernatant; discard pellet).
c) 30 min at 10,000g (save supernatant; discard pellet).
d) Concentrate cell supernatant 10-20 fold in spin concentrator*.

*The quantity of exosomes could vary between samples. A larger starting amount of sample should be used if the signal is weak.

4. Reagent preparation:

a) **Bead Washing Buffer:** Dilute bead washing buffer 5X to 1X with deionized water. 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. Mix 5 ml of 5X Beads Washing Buffer with 20 ml deionized water for a final volume of 25 ml.
b) **RNA Washing Buffer Solution:** 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%.
c) **Elution Buffer and Lysis Buffer are ready to use.**

5. Exosome binding:

a) Purified Exosomes (Lyophilized Standards): Purified exosomes do not require this binding step. If the samples are purified exosomes, skip to RNA extraction directly.

b) Unfractionated Samples:

a. 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 RNA analysis; 0.5 ml up to 1 ml for mRNA analysis.
b. Add 1X PBS to the sample to get a final volume of 1 ml. (If you are using 1 ml of plasma, dilution is not necessary).
c. Add 10 µl of immunobeads.
d. Incubate sample-immunobead mixture overnight at 4°C in a rotator.
e. Centrifuge at room temperature (RT) for 10 min at 5,000g.
f. Discard the supernatant.
g. Wash the beads:
I. Add 1 ml of Bead Washing Buffer.
II. Resuspend up and down 10-15 times.
III. Centrifuge at RT for 10 min at 5,000g.
IV. Remove the supernatant being careful not to disturb the pellet.
V. Wash beads once again as indicated above.

6. RNA Extraction:

a) Lysis:

a. Purified Exosomes: Add 200 µl of Lysis buffer directly to 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 for 5 min at room temperature.
b. Unfractionated Samples: Add 700 µl of Lysis buffer directly on the bead pellet. Dissolve the pellet by pipetting up and down (beads must be totally dissolved). 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 30 sec.
c. Incubate for 10 min at room temperature.
d. Incubate 1 min on ice and centrifuge at 12,000g at 4°C for 10 min. NOTE: Incubation on ice before to centrifuge helps to reduce DNA contamination, which tend to remain in the interphase.
e. Transfer the top phase (aqueous) to a fresh tube.
f. 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 spin column.
a. Spin at 14,000g for 30 sec.
b. Discard the flow-throw.
c. Add the remaining volume into the same spin column.
d. Spin at 14,000g for 30 sec. Discard the flow-throw.

e. Wash column with RNA Washing buffer. Add in the column 400 µl of RNA Washing buffer. Spin at 14,000g for 30 sec. Discard the flow-through. Perform the washing step twice more.
f. Spin 5 additional min at 14,000g to eliminate ethanol residues from column. Discard the flow-throw.
g. Remove the tube and transfer the spin column into an elution tube.
h. Elute the column with 15 µl of Elution buffer. Incubate 5 min at room temperature. Spin 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

7. **Sensitivity:** Purified exosome RNA can be quantified and analyzed using the NanoDrop spectrophotometer, although the measured concentration values are likely to end towards 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 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. miR-21 was amplified by qPCR (Figure 4).

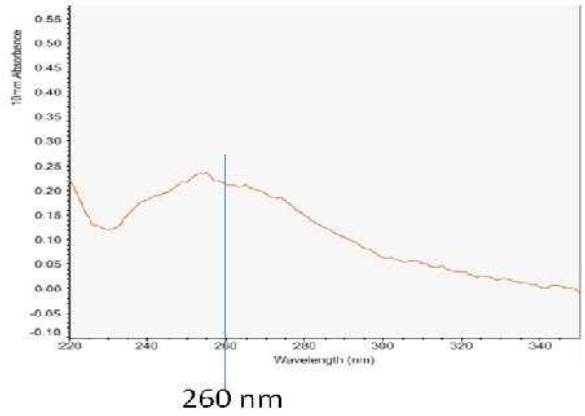

Figure 2: Expected Nanodrop profile for RNA extracted from immunocaptured exosomes (100 µl of human plasma). Yield = 8.4 ng/µl; A260/280 =1.6; A260/230= 1.85.

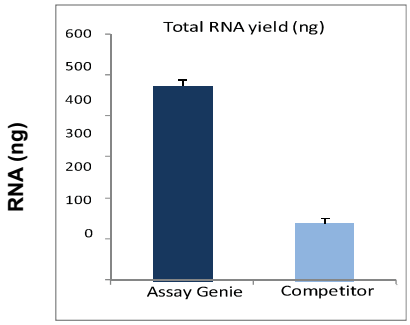

Figure 3. Nanodrop quantification of total RNA yield

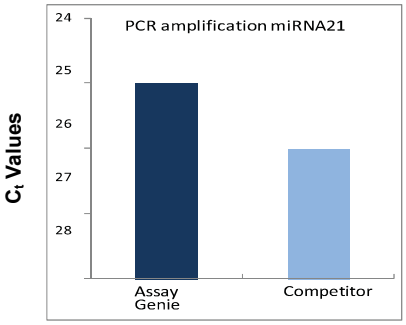

Figure 4. miRNA 21 amplification by qPCR

IX. 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 un-degraded total RNA, it is vital to use the freshest biological material available. Even the 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 the 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 starting material
Little or no total RNA eluted	. Insufficient disruption or homogenization . Incomplete elution	. Reduce the amount of starting material. Overloading reduces yield! . 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 . 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 the Washing Buffer is at room temperature. Check the Washing Buffer for salt precipitates. If there are any precipitates dissolve these precipitates by careful warming

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