Circulating and Exosome-associated DNA Extraction Kit (urine and cell media) (#BN00090)

(Catalog #BN00090 -20, -40; 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. Together with RNAs, genomic single or double-stranded DNA and mitochondrial DNA have been recently detected in exosomes and microvesicles. In particular, the majority of the double-stranded DNA seems to be associated with tumor derived exosomes and can be an important new source of biomarkers for tumor detection.

Isolation and purification of circulating and Exosome-associated genomic DNA combines the ability of Isolation Component to isolate exosomes from urine and cell media with a user-friendly system of DNA purification. Isolated exosomes are lysed with the appropriate lysis buffer and exosome DNA is purified by and exosome DNA is purified by spin columns and optimized buffers with a fast turnaround time. In addition, this kit provides lyophilized exosomes to be used as quality controls for exosome capture and DNA extraction. The procedure takes about 1 hour and 45 minutes for yielding purified genomic DNA. Furthermore, this kit provides lyophilized exosomes to be used as quality controls for exosome capture and DNA extraction. Exosome standards for positive control are also included in the kit. All our kits guarantee high efficiency isolation of circulating and Exosome-associated genomic DNA than similar products.

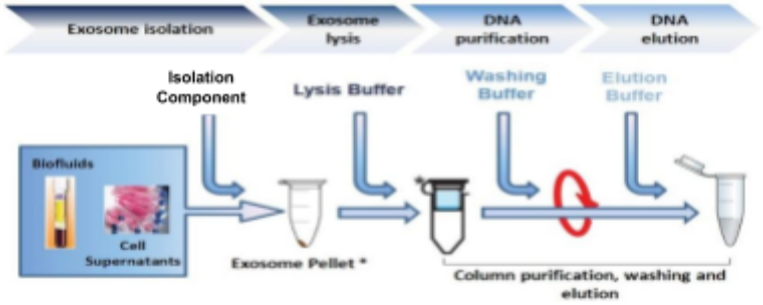

Figure 1. Isolation and purification of circulating and Exosome-associated DNA.

**Pellet of exosomes after isolation can be treated with DNase to eliminate cell free circulating DNA. After DNase treatment DNA extraction from microvesicle proceeds following the same protocol.*

II. Application:

- Direct exosome capture and DNA purification from urine and cell media without time consuming purification steps.
- Isolation of genomic exosome-associated DNA by DNase treatment.
- Profiling of exosome associated genomic DNA.
- Purified genomic DNA is ready for downstream analysis such as PCR, RT-PCR, qRT-PCR and microarrays.

III. Sample Type:

- Human biological fluids including Urine and Cell Media.

IV. Kit Contents (for Isolation of circulating and Exosome-associated DNA) (from urine and cell media):

Components	BN00090	BN00090	Part Number
	20 assays	40 assays	
Isolation Component	1 bottle (21 ml, 20 reactions)	2 bottles (2 x 21 ml, 40 reactions)	BN00090-1
Lysis buffer	1 bottle (5 ml)	1 bottle (9 ml)	BN00090-2
Proteinase K	1 vial (450 µl)	2 vials (450 µl)	BN00090-3
Washing buffer 1	1 bottle (6 ml, to add 10 ml of ethanol 96%)	2 bottles (6 ml, to add 10 ml of ethanol 96%)	BN00090-4
Washing buffer 2	1 bottle (5 ml, to add 12 ml of ethanol 96%)	2 bottles (5 ml, to add 12 ml of ethanol 96%)	BN00090-5
Elution buffer	1 vial (1.5 ml)	1 vial (1.5 ml)	BN00090-6
Columns	22 columns	42 columns	BN00090-7
RNase free elution tubes (1.5 ml)	22 tubes	42 tubes	BN00090-8
Lyophilized Exosome Standard (Urine/Cell media)	1 vial (100 µg)	1 vial (100 µg)	BN00090-9

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%

VI. Shipment and Storage:

All the reagents are shipped and stored at +4°C for up to 12 months, if unopened. Briefly centrifuge small vials prior to opening. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- The RNase free column and elution tubes should be stored at room temperature as well as at 4°C.
- The remaining reconstituted Exosome standard stock solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze and thaw cycles.
- All the opened buffers and diluted reagents including Isolation Component, lysis buffer, washing buffers, ploy A carrier, proteinase K and the elution buffer should be stored at 4°C.

VIII. Assay Protocol:

1. **Urine sample preparation:** Centrifuge 10 min at 350 g at RT to eliminate cells and protein aggregates. Save the supernatant.
2. **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).

3. Reagent preparation:

a) Washing Buffer1 and Washing buffer 2: Add the volume of pure ethanol (96%) indicated on the label of the bottles of both Washing Buffers.

b) Isolation Component, Elution buffer and Lysis buffer are ready to use.

4. Lyophilized Exosome Standards:

a) Reconstitute lyophilized exosome standard by adding 100 µl of deionized water and pipetting the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted standard for 60 sec. Briefly centrifuge the tubes containing the standard to ensure that the solution is collected at the bottom of the tube.

5. **Exosome isolation from Urine:**

a) Add 1 ml of Isolation Component to 4 ml of precleared urine.
b) Mix well by pipetting and inverting tube.
c) Incubate on ice for 1 hr.
d) Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C or at RT)
e) Discard the supernatant.
f) Centrifuge for 2 min at 1500g to eliminate entirely the supernatant.
g) Resuspend isolated exosomes in 100 µl of 1X PBS.

6. **Exosome isolation from Cell Media:**

a) Add 1 ml of Isolation Component reagent to 1 ml of precleared cell medium.
b) Mix well by pipetting and inverting tube.
c) Incubate on ice for 1 hr.
d) Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C or at RT).
e) Discard the supernatant.t
f) Centrifuge for 2 min at 1500g to eliminate entirely the supernatant.
g) Resuspend the pellet in 100 µl of 1X PBS.

7. **DNA Extraction:**

a) Lysis:

a. Add 20 µl of Proteinase K (600 mAU/ml).
b. Add 200 µl of Lysis Buffer.
c. Add 5 ul of Poly A Carrier (optional)*.
d. Mix well by vortexing 30 sec.
e. Incubate samples at 56°C for 10 min.

* The use of Poly A Carrier results in an increase in DNA yield, in particular when small amounts of DNA are purified through silica membrane columns. No adverse effect on downstream applications such as PCR have been observed (Shaw et al. 2009).

b) **DNA Purification:**

a. Add 200 ul of Ethanol 96% and mix by inverting the tube 6-8 times.
b. Transfer the mixture in a Spin Column and centrifuge at 10,000 g for 1 min. Discard the flow-through.
c. Add 500 µl of Washing Buffer 1, centrifuge for 1 min and discard the flow-through.
d. Add 500 µl of Washing Buffer 2, centrifuge for 1 min and discard the flow-through.
e. Centrifuge 2 additional min at 16,000g.
f. Transfer the spin column to an Elution Tube.
g. Elute the DNA from the column adding 50 µl of Elution Buffer.
h. Incubate for 5 min at room temperature.
i. Centrifuge 1 min at 200g.
j. Centrifuge 1 min at 16,000g.

8. **Sensitivity:** This kit guarantees high efficiency isolation of circulating and Exosome-associated DNA.

Ct Values (Fluorescence)

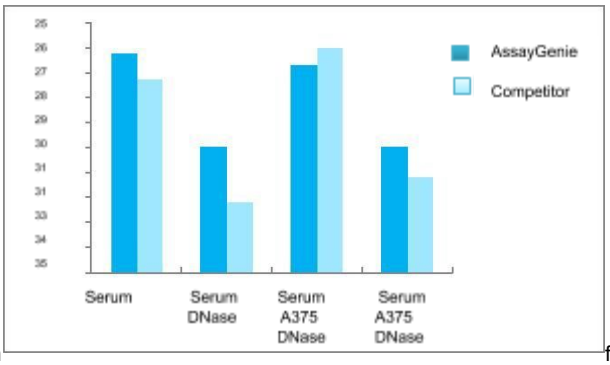

Figure 2. Amplification of beta-actin from exosome-derived DNA. Exosomes were isolated from serum with our without artificial spike (A375-derived exosomes) using Exosome Purification solution and treated (or not) with DNase I. DNA was extracted with the AssayGenie kit and competitor and beta actin was amplified by qPCR.

IX. General Troubleshooting Guide:

Problems/Cause	Solution
Low DNA Yield	. Be sure to add Proteinase K in the mixture of lysis buffer . Increase the incubation at RT during the elution step . Do not use water to elute DNA but use only the Elution buffer provided in the kit . Avoid to mix the lysate too vigorously . Avoid to form bubbles during mixing steps
DNA is sheared or degraded	. Do not touch the membrane of the column with the tip . Treatment with DNase must be done before to lyse the vesicles. Be careful to deactivate the DNase before to proceed to the lysis . Avoid repeated freeze and thaw cycles
Incomplete elution	. Prolong the incubation time with Elution Buffer to 5-10 min or repeat elution step once again (25 µl + 25 µl)
Ethanol Contamination	. After the second washing step, centrifuge once again for 2 min at 15,000g. Dry the membrane of the column by incubation at RT (no flow hood)

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