ExoDNAPS™ Circulating and Exosome-associated DNA Extraction Kit (plasma/serum)

(Cat# *K1230-20; Store at 4°C) *(*Not available for sale in USA)*

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. Circulating DNA is emerging as a novel non-invasive tool for patient's stratification and disease monitoring. While most of the research has focused on circulating cell-free (cfDNA) or circulating-tumor-cell (CTC) derived DNA, extracellular vesicle (EVs) associated DNA (EV-DNA) is emerging as a third valuable "liquid biopsy" platform. 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.

ExoDNAPS™ Kit (isolation and purification of circulating and Exosome-associated DNA) combines the ability of our ExoPure™ reagent to isolate EVs and circulating DNA from biofluids (plasma and serum) with a user friendly system of DNA purification. Isolated vesicles are lysed with the appropriate lysis buffer and DNA is purified by and exosome DNA is purified by spin columns and optimized buffers with a fast turnaround time (~30 min). The kit provides an appropriated DNA concentrator for concentrating the yield (4-fold concentration) and increasing the purity of the DNA to the levels required for digital PCR analysis.

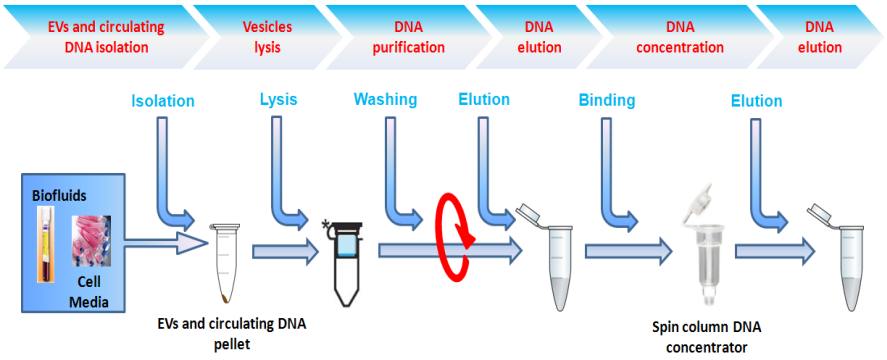

Figure 1. ExoDNAPS™ isolation and purification of circulating and Exosome-associated DNA.

** Analysis can be limited to EV-associated DNA by treating the pellet containing circulating DNA and EVs with DNAse. Extracellular vesicles protect the internal DNA from the DNAse digestion.*

II. Applications:

- Isolation and profiling of genomic EV-associated DNA by DNAse treatment.
- Direct exosome capture and DNA purification from biofluids or cell media without time consuming EV purification steps
- Discovering of mutations by QPCR and digital PCR analysis
- DNA suitable for NGS.

III. Sample Type:

- Human biological fluids including Plasma and Serum.

IV. Kit Contents (for Isolation of circulating and Exosome-associated DNA) (from human plasma and serum):

Components	K1230-20 20 Assays	Part Number
ExoPure™ Reagent	1 bottle (3 ml)	K1230-XX-1
Lysis buffer	1 bottle (5 ml)	K1230-XX-2
Proteinase K	1 vial (450 µl)	K1230-XX-3
Washing buffer 1	1 bottle (6 ml, to add 10 ml of ethanol 96%)	K1230-XX-4
Washing buffer 2	2 bottles (5 ml, to add 12 ml of ethanol 96%)	K1230-XX-5
Elution buffer	1 vial (1.5 ml)	K1230-XX-6
Purification Columns	22 columns	K1230-XX-7
RNAse free elution tubes (1.5 ml)	22 tubes	K1230-XX-8
Lyophilized Exosome Standard (Plasma/Serum)	1 vial (100 µg)	K1230-XX-9
Binding Buffer	1 vial (1.5 ml)	K1230-XX-10
DNA Concentration Columns	22 columns	K1230-XX-11

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 columns and elution tubes should be stored at room temperature as well as at 4°C.
- Washing Buffer 1 and Washing buffer 2: Add the volume of pure ethanol (96%) indicated on the label of the bottles of both Buffers.
- Lyophilized Exosome Standards: 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. The 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.
- ExoPureTM Reagent, Elution buffer, Binding buffer, Washing buffer 1, Washing Buffer 2, Proteinase K and Lysis buffer are ready to use and should be stored at 4°C.

VIII. ExoDNAPS™ Assay Protocol:

1. Plasma and Serum sample preparation: Prepare plasma/serum 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; optional step to eliminate vesicles > 200 nm)

2. Exosome isolation (from Plasma and Serum):

a) Add 125 µl of ExoPure™ Reagent (ratio Isolation Component/Sample 1/4) to 500 µl of precleared sample.
b) Mix well by pipetting and inverting the tube.
c) Incubate on ice for 1 hr.
d) Centrifuge 20 min at 10,000g (centrifuge can be performed at 4°C as well as at RT).
e) Discard the supernatant.
f) Resuspend the isolated exosomes in 200 µl of 1X PBS.

3. DNA Extraction:

a) Lysis:

i. Add 20 µl of Proteinase K (600 mAU/ml).
ii. Add 200 µl of Lysis Buffer.
iii. Mix well by vortexing 30 sec.
iv. Incubate samples at 56°C for 10 min.

b) DNA Purification:

i. Add 200 ul of Ethanol 96% and mix by inverting the tube 6-8 times.
ii. Transfer the mixture in a Purification Spin Column and centrifuge at 10,000 g for 1 min. Discard the flow-through.
iii. Add 500 µl of Washing Buffer 1, centrifuge for 1 min and discard the flow-through.
iv. Add 500 µl of Washing Buffer 2, centrifuge for 1 min and discard the flow-through.
v. Centrifuge 2 additional min at 16,000g.
vi. Transfer the spin column to an Elution Tube.
vii. Elute the DNA from the column adding 50 µl of Elution Buffer.
viii. Incubate for 5 min at room temperature.
ix. Centrifuge 1 min at 200g.
x. Centrifuge 1 min at 16,000g.

c) DNA Concentration:

i. Add 50 µl of Binding buffer to eluted DNA.
ii. Mix well by pipetting.
iii. Add 200 µl of Ethanol 96% and mix by inverting the tube 6-8 times.
iv. Transfer the mixture in the DNA Concentration Column and centrifuge at 14000g for 1 min. Discard the flow-through.
v. Wash by adding 500 µl of Washing Buffer 2, centrifuge for 1 min at 14000g and discard the flow-through.
vi. Repeat the washing step once.
vii. Centrifuge 2 additional min at 14000g.
viii. Elute the DNA from the column adding 10 µl of Elution Buffer.
ix. Incubate for 5 min at room temperature.
x. Centrifuge 1 min at 16,000g.

IX. Sensitivity:

Exosome-associated DNA is suitable for point mutation analysis by allele-specific PCR

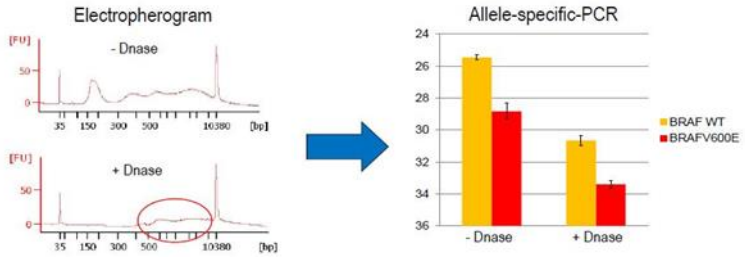

Figure 2. Healthy donor serum was spiked with 100 µg of purified exosome from BRAFV600E-positive A375 melanoma cell lines. Vesicles and circulating DNA were isolated by precipitation with ExoPure™ and treated with or without DNAse 1, to distinguish circulating + EV related and only EV related DNA. Following digestion, DNA was extracted with ExoPure™ Kit and analyzed by bioanalysis and allele-specific QPCR.

ExoDNAPS™ kit guarantees high efficiency isolation of circulating and Exosome-associated DNA

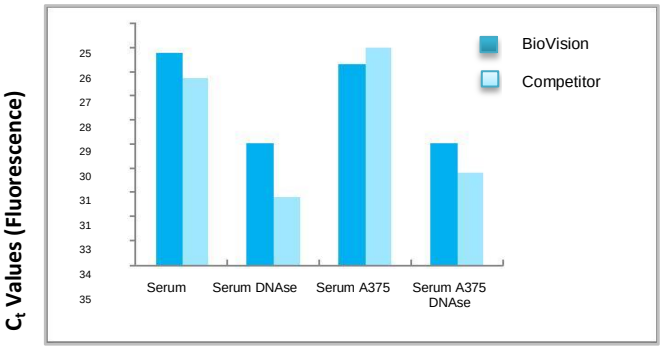

Figure 2. Amplification of beta-actin from exosome-derived DNA. Exosomes were isolated from serum with our without artificial spike (A375-derived exosomes) using ExoPure™ solution and treated (or not) with DNAse I. DNA was extracted with ExoDNAPS™ kit and competitor and beta actin was amplified by qPCR.

X. Related Products:

Products/Catalog Number
ExoDNAPS™ circulating and Exosome associated DNA from plasma and serum # *K1230-20
ExoDNAPS™ circulating and Exosome associated DNA from plasma and serum # *K1230-40
ExoDNAUC™ circulating and Exosome associated DNA from urine and cell media # *K1231-20
ExoDNAUC™ circulating and Exosome associated DNA from urine and cell media # *K1231-40

XI. 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
DNA is sheared or degraded	• Avoid mixing the lysate too vigorously • Avoid forming bubbles during mixing steps • 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 15,000 g. Dry the membrane of the column by incubation at RT (no flow hood)

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