Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

ExoDNAPS™ Conc Exosome DNA Extraction Kit

(Cat# *K1243-40; Store at 4°C; Optimized protocol for dPCR and NGS) ***(*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 the molecular composition of released exosomes depend on the state of a parent cell. 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 extracellular vesicles. However, the majority of double-stranded DNA seems to be associated with tumor derived exosomes (Thakur BK et al. 2014; Kalhert et al. 2014) where it represents the entire genome of the cancerous tumor from which exosomes were derived. This discovery corroborates the potential of exosomes, which can be easily obtained from a simple blood test.

ExoDNAPS™ Conc Kit combines the ability of our reagent ExoPure™ to co-isolate EVs and circulating DNA from biofluids (plasma, urine, serum etc.) or culture supernatants with a user friendly system for DNA purification. Isolated vesicles are lysed with the appropriate lysis buffer and DNA is purified by spin columns and optimized buffers with a fast turnaround time (≈30 min). Finally, ExoDNAPS™ Conc 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 (dPCR).

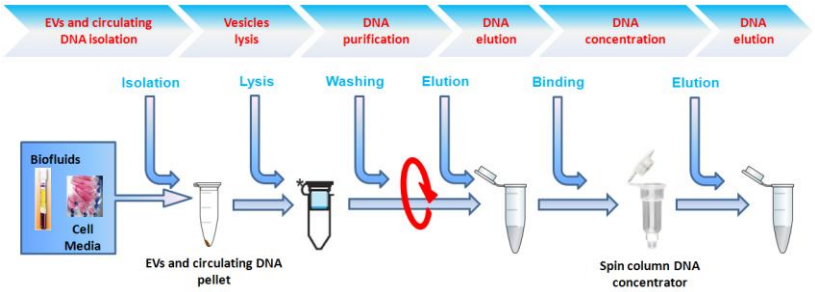


Figure 1. ExoDNAPS™ Conc Isolation and Purification of Circulating and Exosome-Associated DNA

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

II. Application:

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

III. Advantages:

- Highly pure circulating and EVs-associated DNA
- Fast and easy protocol
- Small volume amount required
- Suitable for concomitant profiling of cell-free circulating DNA and EV-associated DNA

IV. Sample Type:

- Human biological fluids including Plasma and Serum

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

Components	K1243-40 40 Assays	Part Number
ExoPure™ Reagent	1 bottle (5 ml, 40 reactions)	K1243-40-1
Lysis buffer	1 bottle (9 ml)	K1243-40-2
Washing buffer 1	2 bottles (6 ml, to add 10 ml of ethanol 96%)	K1243-40-3
Washing buffer 2	2 bottles (5 ml, to add 12 ml of ethanol 96%)	K1243-40-4
Elution buffer	1 vial (1.5 ml)	K1243-40-5
Purification Columns	42 columns	K1243-40-6
Elution tubes (1.5 ml)	42 tubes	K1243-40-7
Binding Buffer	2 vials (1 ml each)	K1243-40-8
DNA Concentration Columns	42 columns	K1243-40-9
Lyophilized Exosome Standard (Plasma/Serum)	1 vial (100 µg)	K1243-40-10
Proteinase K	2 vials (450 µl each)	K1243-40-11

VI. 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%

VII. 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!

VIII. Reagent Preparation and Storage Conditions:

- Washing Buffer 1 and Washing buffer 2: Add the volume of pure ethanol (96%) indicated on the label of the bottles of both Buffers.

- ExoPure™, Elution buffer, Binding buffer and Lysis buffer are ready to use and should be stored at 4°C.
- Columns and Elution tubes can be stored at room temperature as well as at 4°C
- 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 remaining reconstituted 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.

IX. ExoDNAPS™ Conc Assay Protocol:

1. Plasma and Serum Sample Preparation: Starting Volume (500 µl): 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)

2. Reagent Preparation:

a) Washing Buffer 1 and Washing buffer 2- Add the volume of pure ethanol (96%) indicated on the label of the bottles of both Washing Buffers.
b) ExoPure™, Elution buffer and Lysis buffer are ready to use.

3. 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.

4. Exosome isolation (from Plasma and Serum):

a) Add 125 µl of Isolation Component (ExoPure™) (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) Eliminate the remaining supernatant into the tube with a tip.
g) Resuspend isolated exosomes in 200 µl of 1x PBS.

5. DNA Extraction:

a) Lysis:

a. Add 20 µl of Proteinase K.
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 a 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 µl 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 400 µl of Washing Buffer 1, centrifuge for 1 min and discard the flow-through.
d. Add 400 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.

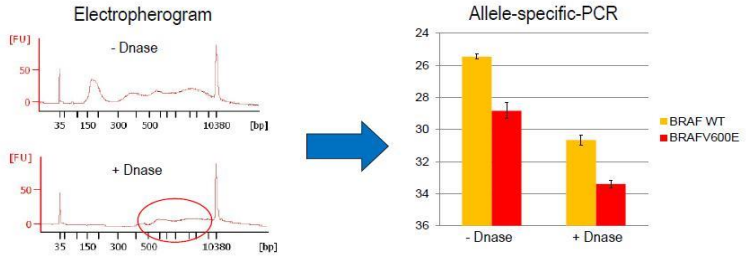
c) DNA Concentration:

a. Add 50 µl of Binding buffer to eluted DNA.
b. Mix well by pipetting.
c. Add 200 µl of Ethanol 96% and mix by inverting the tube 6-8 times.
d. Transfer the mixture in the DNA Column Concentrator and centrifuge at 14000g for 1 min. Discard the flow-through.
e. Wash by adding 400 µl of Washing Buffer 2, centrifuge for 1 min at 14000g and discard the flow-through.
f. Centrifuge 2 additional min at 14000g.
g. Elute the DNA from the column adding 10 µl of Elution Buffer.
h. Incubate for 5 min at room temperature.
i. Centrifuge 1 min at 16000g.

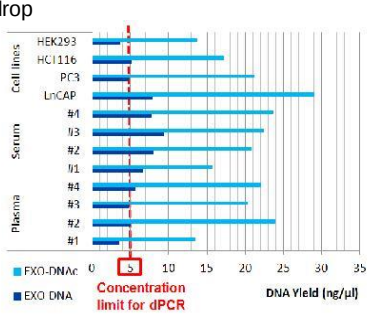
X. Sensitivity: Exosome-associated DNA is suitable for point mutation analysis by allele-specific PCR:

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 (Fig 2 and 3).

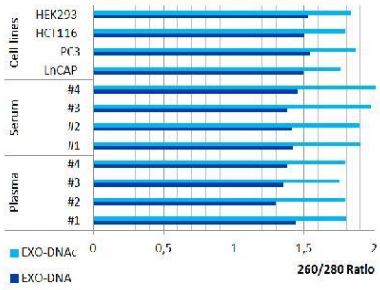
Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com



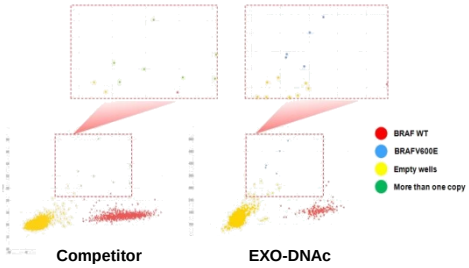
Concentrator improves the quality of circulating and EV-associated DNA, suitable for dPCR and NGS:
Circulating and EVs associated DNA was extracted from different samples, including human plasma, serum and four cell culture media using both ExoDNA™ (EXO-DNA) and ExoDNA™ Conc (EXO-DNAc), optimized with the concentrator columns. DNA yields were finally analyzed by Agilent Bioanalyzer and by Nanodrop



ExoDNA™ Conc Kit (EXO-DNAc) is able to concentrate around 4X the DNA yield extracted with the standard protocol (EXO-DNA). DNA yield measured by Nanodrop.



ExoDNA™ Conc Kit (EXO-DNAc) improves the DNA purity, compared to the standard protocol (EXO-DNA). DNA purity is expressed by 260/280 ratio.



Detection of the mutation BRAFV600E by digital PCR in genomic DNA isolated from serum of a metastatic prostate cancer patient treated with abiraterone. Circulating and EVs associated DNA was extracted by ExoDNA™ Conc Kit (EXO-DNAc) and a competitor kit. Digital PCR analysis revealed mutations in DNA extracted with (EXO-DNAc), whereas no mutation was detectable in DNA extracted with competitor kit. (Validated by Exosomics Siena).

XI. Related Products:

Product Name	Cat. No.
ExoDNAPS™ Circulating and Exosome associated DNA (plasma/serum)	*K1230
ExoDNAPS™ Conc Exosome DNA Extraction Kit (plasma/serum)	*K1243
ExoDNAUC™ Circulating and Exosome associated DNA (urine/cell media)	*K1231
ExoDNAUC™ Conc Exosome DNA Extraction Kit (urine/cell media)	*K1244
ExoDNA™ Extraction Kit	K1242

Not available for sale in USA

XII. Trouble Shooting:

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.