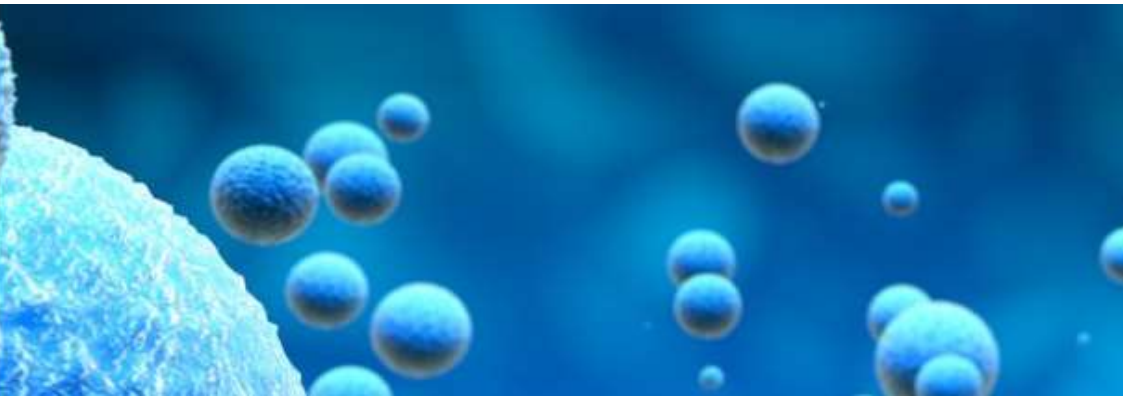

SeleCTEV EV-DNA Enrichment kit

Low Volume

Cat.N. EXO-SEL-LV

www.exosomics.it

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1. PRODUCT OVERVIEW

EXOSOMICS SeleCTEV™ Enrichment kit is an innovative workflow to selectively purify tumor-originated nucleic acids from tumor enriched extracellular vesicles (EVs) and exosomes from biofluids. The purification is based on Exosomics proprietary peptide affinity method and does not require any special equipment, such as ultracentrifugation or chromatography.

The **SeleCTEV™ EV-DNA Low Volume (EXO-SEL-LV)** kit is versatile: users can choose from different biofluid input volumes (ranging from 0.5 ml up to 2 ml) and DNA-specific workflows.

- **DNA extraction package** is designed by Exosomics experts to co-isolate EV- and circulating cell-free DNA (cf-DNA) from plasma and/or serum of the patient with a user-friendly protocol for DNA purification. While most of the research has focused on circulating cell-free tumor (ctDNA) or circulating-tumor-cell-(CTC)-derived DNA, EV-associated DNA (EV-DNA) is emerging as a third valuable “liquid biopsy” platform.

KIT SPECIFICATIONS

SeleCTEV™	Cat. number	Volume	Biofluid	Extraction kit
Low Volume-DNA	EXO-SEL-LV	0.5 -2 ml	Plasma, serum	cfDNA and EV-DNA

FEATURES AND BENEFITS

Unique	This kit is designed to selectively purify tumor-originated nucleic acids from tumor enriched extracellular vesicles and exosomes from biofluids.
Fast and Accurate	No time-consuming ultracentrifugation step needed, turnaround time is minimum of 4 hours.
Versatile	Users can choose from different biofluid, input volumes (ranging from 0.5 ml up to 2 ml) and DNA-specific workflows.

2. PRINCIPLES OF THE PROCEDURE

The SeleCTEV™ Enrichment kit is ready-to-use and it is meant for running 20 tests. Kit allows the selective isolation of tumor-originated nucleic acids from tumor enriched EVs and exosomes, from a minimum of 500 µl of plasma/serum following two subsequent working steps as depicted below:

- Cell free circulating and EV-associated DNA isolation from biofluid of patient.
- DNA purification.

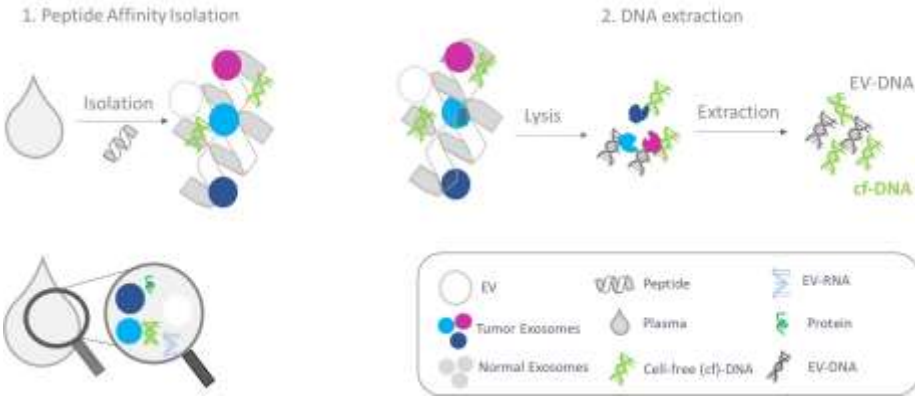

Figure 1 SeleCTEV™ Isolation and EV-DNA Extraction kit

3. KIT COMPONENTS AND STORAGE

3.1 Kit Components:

Component	Name	Description	Amount	Storage
Isolation Agent	EXO-IA	Lyophilized Reagent for Isolation	1 vial (2 mg)	4°C
Resuspension Buffer	EXO-RB	Peptide resuspension buffer	1 vial (1 ml)	4°C
Isolation Buffer (10X)	EXO-IB	Diluent for Isolation	1 bottle (30 ml)	RT
Isolation Tubes	EXO-IsoT-2ml	Tubes for EV Isolation	40 tubes (2 ml)	RT
Proteinase K	EXO-PK	Reagent for protein Digestion	1 ml (20mg/ml)	4°C
Lysis Buffer	EXO-LB	Solution for vesicle Lysis	2 bottles* (4 ml)	RT
Washing Buffer 1	EXO-WB1	Solution for column Washing	1 bottles (15 ml)	4°C
Washing Buffer 2	EXO-WB2	Solution for column Washing	1 bottles (15 ml)	4°C
Elution Buffer	EXO-EB	Solution for DNA Elution	1 bottle (4 ml)	RT
DNA purification columns	EXO-DC	Columns for DNA purification	20 Columns	RT
Elution Tubes	EXO-ColT	Tubes for pure DNA collection	20 Elution tubes (1.5ml)	RT

***** An extra bottle of LB is supplied for sample volume of 2 ml.

Customer supplied reagents and equipment:

- Protease inhibitor (Sigma cat num. P8340)
- Ethanol 96-100%
- Disposable Gloves
- Single-use and/or pipettes with disposable tips
- Pipettes for reagent preparation
- MilliQ water
- Heating block, or water bath for incubation at 56°C
- Benchtop centrifuge with rotor for 2 ml or 15 ml reaction tubes
- Vortex

3.2 Storage Conditions: The SeleCTEV™ kit is shipped at controlled temperature (4-8°C) with ice packs. All components must be stored carefully according to the indication in the table below. Properly sealed reagents are stable at the indicated storage temperature for at least 12 months after kit delivery.

4. HANDLING OF BLOOD AND PLASMA

Note: Suggested procedure for blood collection, and plasma/serum processing can be requested at info@exosomics.eu

Transport and storage

Plasma and serum samples must be shipped in dry ice and stored at -80°C. Aliquoting is recommended since freeze-and-thaw cycles reduce the quality of the sample.

5. PROCEDURE FOR EXOSOME ISOLATION AND DNA EXTRACTION

Each test requires at least 500 µl of plasma or serum. Volumes can be scaled up to 2 ml (SeleCTEV™ Low Volume, cat.n.EXO-SEL-LV)

or 7 ml (SeleCTEV™ High Volume, cat.n. EXO-SEL-HV), according to sample availability.

Sample Volumes: SeleCTEV™ Low Volume has been optimized for sample volumes ranging from 0.5 ml to 2 ml of plasma or serum. Follow steps 1-4 up to 1 ml of plasma/serum. For 2 ml of plasma/serum, the best performance is obtained by splitting plasma/serum into two 2 ml vials (EXO-IsoT-2ml) and then proceeding through steps 1-4 as for 1 ml samples.

1 Plasma/Serum preparation:

1.1 Pre-clear the plasma or serum sample by centrifuging at 1200 g for 20 min at 10°C to eliminate red blood cells and cellular debris.

1.2 Discard the pellet and debris and transfer the supernatant in the appropriate tube (EXO-IsoT-2ml).

1.3 Dilute 10X Isolation Buffer (EXO-IB) in fresh milliQ water to a final 1X concentration (i.e. 1 ml of EXO-IB and 9 ml of mQ water), and label the vial as “1X-IB”.

1.4 Dilute pre-cleared plasma or serum in 1:1 v/v with 1X-IB (i.e. If used 0.5 ml of plasma, add 0.5 ml of IB). If processing 2 ml of plasma/serum, split the sample into two 2 ml isolation tubes (EXO-IsoT-2ml) and dilute 1 ml of pre-cleared plasma with 1 ml of 1X-IB.

1.5 Add protease inhibitor cocktail to each sample (1:1000 v/v protease: diluted plasma. Not provided with the kit, we recommend Sigma cat num. P8340.)

2 Reagent preparation:

2.1 **Isolation Agent:** add 800µl of Resuspension Buffer (EXO-RB) into the Isolation agent (EXO-IA) vial. Gently tap the vial and visually check for complete resuspension of the lyophilized reagent. Do not pipet up and down.

2.2 **Washing Buffer 1 (WB1):** add 9,4 ml of pure Ethanol (96-100%) in EXO-WB1 bottle (15 ml). Mix well by inverting 6-8 times.

2.3 **Washing Buffer 2 (WB2):** add 10,5 ml of pure Ethanol (96-100%) to EXO-WB2 bottle (15 ml). Mix well by inverting 6-8 times.

3 Cell-free DNA and EV isolation from plasma or serum:

3.1. Add 20µl of resuspended Isolation agent (EXO-IA) to each vial of pre-cleared diluted sample.
3.2. Mix well by pipetting and inverting the tube.
3.3. Incubation time is 2 hours at RT under rotation.
3.4. Centrifuge 15 min at 16000 g at RT.
3.5. Discard the supernatant, carefully avoiding to dislodge the pellet. Eliminate the remaining supernatant from the tube with a pipette.
3.6. Gently add 1ml of 1X Isolation Buffer (1X-IB) directly on the pellet, without disrupting it. Spin the sample at 7000 g for 7 min at RT.

Note: If the pellet is not visible at this step, refer to Technical Support (Section 7, p.11).

3.7. Repeat steps 3.5-3.6 one more time.
3.8. Resuspend the (each) pellet in 200 µl of Isolation Buffer (1X-IB).

Note: we advise to proceed directly to step 4 (DNA purification) to obtain optimal DNA recovery.

4 DNA purification:

4.1 EV Lysis:

4.1.1 Add 20 µl of Proteinase K (20mg/ml), (EXO-PK), to each resuspended pellet and mix by gently vortexing the tube.
4.1.2 Add 200 µl of Lysis Buffer (EXO-LB) to each tube (i.e. if processing 2 ml of plasma, add 200 µl of EXO-LB to each tube).

Note: an extra bottle of Lysis Buffer is supplied for plasma volume of 2 ml.

4.1.3 Mix well by vortexing 30 sec.
4.1.4 Incubate samples at 56°C for 1 hour.

4.2 DNA purification:

4.2.1 Add 200 µl of Ethanol 96-100% to each tube and mix by briefly vortexing the tube.
4.2.2 Transfer the mixtures in a DNA Spin Column (EXO-DC) and centrifuge at 10000 g for 1 min.

Note: if processing 2 ml of plasma, repeat steps from 4.1.1 to 4.2.1 with two tubes, and then load them into the same DNA Spin Column (4.2.2).

4.2.3 Discard the flow-through.
4.2.4 Add 500 µl of Washing Buffer 1 (EXO-WB1), centrifuge at 10000 g for 1 min and discard the flow-through.

4.2.5 Add 500 µl of Washing Buffer 2 (EXO-WB2), centrifuge at 10000 g for 1 min and discard the flow-through.
4.2.6 Centrifuge 2 additional min at 16000 g.
4.2.7 Transfer the spin column to an Elution Tube (EXO-ColT).
4.2.8 Elute the DNA from the column adding 50 µl of Elution Buffer (EXO-EB).
4.2.9 Incubate for 5 min at RT.
4.2.10 Centrifuge 1 min at 14000 g. Samples can now be used for further analyses or stored at -20°C.

The SeleCTEV EV-DNA Enrichment Procedure (Low Volume)

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6. RELATED PRODUCTS

Exosomics Related Products

Kit	Cat. Number	Volume	Biofluid	Extraction kit
SortEV™ Enrichment kit	EXO-SOR-LV	0.5 ml -2 ml	Plasma, serum	EV-RNA
SeleCTEV™ High Volume-Enrichment kit	EXO-SEL-HV	>2 ml - 7 ml	Plasma, serum	cfDNA and EV-DNA

7. TECHNICAL SUPPORT

This table may solve some technical problems that could arise during SeleCTEV protocol execution. For more information, please contact us at info@exosomics.eu.

Technical Problems	Potential Causes	Suggestions and comments
Poor DNA recovery	Use of anticoagulants other than EDTA may not fully preserve circulating DNA. Repeat blood collection according to Exosomics' protocol	Please request your copy of Exosomics supportive protocols at info@exosomics.eu.

Poor DNA recovery	Poor plasma quality due to delayed blood processing. Repeat blood processing to plasma according to Exosomics' protocol	Please request your copy of Exosomics supportive protocols at info@exosomics.eu.
	Plasma samples are frozen and thawed multiple times	Always use fresh samples or samples thawed once.
	Prolonged sample storage at room temperature	Do not keep the samples at RT for prolonged time.
	Incomplete resuspension of the peptide	Peptide solution may initially look cloudy after resuspension in resuspension buffer (EXO-RB). Do not vortex the solution, simply tap the vial to resuspend the peptide. Make sure that the peptide is fully resuspended in EXO-RB and the final solution looks clear.
	No visible pellet	It may occasionally occur but should not affect DNA recovery.
	Lysis buffer (EXO-LB) and pellet-proteinase K solutions not sufficiently mixed	Mix lysis buffer (EXO-LB) and pellet-proteinase K solution well by pipetting up and down and vortexing at least 30'' to completely resuspend the peptide pellet.
	Inefficient sample lysis	Use fresh proteinase K. If needed, increase

Poor DNA recovery		incubation time with proteinase K.
	Sub-optimal ethanol percentage	Use fresh 96-100% ethanol. Do not use denatured alcohol which may contain methanol.
	Clogged DNA spin column	Repeat the procedure increasing the incubation time in proteinase K.
	Wash buffers 1 and 2 (EXO-WB1; EXO-WB2) prepared incorrectly	Check that these buffers were diluted in the correct volume of 96-100% ethanol (see page 7).
	The eluate volume is lower than the applied volume	Expect to recover an eluate volume with 2-3 µl less than the applied volume due to retention of the silica membrane.
DNA not suitable for enzymatic reaction	Presence of ethanol traces in eluate	Make sure to remove all ethanol residuals from the column (EXO-DC) before eluting the sample.
	Extremely low or no DNA recovered	See poor DNA recovery section above for troubleshooting.
	Not optimized elution volume	Calculate the optimal elution volume for PCR reaction.
	New PCR assay	If the PCR assay is changed, readjust the eluate volume.
	Interference due to plasma inhibitors	Consider the presence of plasma inhibitors such as natural or synthetic small molecule (therapeutics)

		that may end up in the eluate and inhibit DNA amplification.

SeleCTEV-DNA

Low Volume

Cat.N: EXO-SEL-LV

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