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SeleCTEV™ DNA Low Volume Enrichment Kit

User Manual – Version 2018/09

Σ 24 reactions

REF EXO-SEL-LV

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Intended Use

SeleCTEV™ EV-DNA Enrichment kit is a pre-analytical kit to purify both circulating cell free DNA and tumor-originated DNA from tumor enriched extracellular vesicles (EVs) and exosomes from plasma.
The **SeleCTEV™ EV-DNA Low Volume** (EXO-SEL-LV) kit is optimized for input volumes ranging from 0.5 ml up to 2 ml of plasma.

The kit is designed to be used with any downstream application employing enzymatic amplification or enzymatic modifications of DNA isolated with SeleCTEV, followed by signal detection or amplification (*in vitro* diagnostic applications such as PCR, real time PCR and digital PCR). Any diagnostic results generated using DNA isolated with **SeleCTEV™ EV-DNA** Enrichment kit in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

Exosomics **SeleCTEV™ EV-DNA** Enrichment kit does not provide a diagnostic result. It is responsibility of the user to use and validate the kit in conjunction with any downstream *in vitro* diagnostic assay.

Product Description

SeleCTEV™ DNA Enrichment kit is ready-to-use and it is meant for running 24 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 following two subsequent working steps:

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

The purification is based on Exosomics proprietary peptide affinity method and does not require any special equipment, such as ultracentrifugation or chromatography, with a turnaround time of a minimum of 4 hours.

Materials Provided/Required

Kit components, meant to run a total of 24 reactions, and their storage conditions are listed below table.

Kit components

Product Code (Input Volume)			**EXO-SEL-LV (0,5-2ml)**
Components	**Name**	**Description**	**Quantity (volume/number)**
Isolation Agent	EXO-IA	Lyophilized Reagent for isolation	1 vial (2.5 mg)
Resuspension Buffer	EXO-RB	EXO-IA Resuspension Buffer	1 vial (1 ml)
10X Isolation Buffer	EXO-IB	Diluent for Isolation	1 bottle (30 ml)
Isolation Tubes	EXO-IsoT-2ml	Tubes for EV isolation	48 tubes (2 ml)
Proteinase K	EXO-PK	Reagent for Protein Digestion	1 ml (20 mg/ml)
Lysis Buffer	EXO-LB	Buffer for vesicle Lysis	1 bottle (10 ml)
Washing Buffer 1	EXO-WB1	Buffer for column Washing	1 bottle (15 ml)
Washing Buffer 2	EXO-WB2	Buffer for column Washing	1 bottle (15 ml)

Elution Buffer	EXO-EB	Buffer for DNA elution	1 bottle (4 ml)
DNA purification columns	EXO-DC	Columns for DNA Elution	24 columns
Elution Tubes	EXO-ColT-1.5 ml	Tubes for pure DNA collection	24 collection tubes (1.5 ml)

Store all the supplied reagents according to the instructions on the respective packages. In particular:

- Upon EXO-IA resuspension in EXO-RB, store the vial at +2/+8 °C;
- Upon preparation of EXO-WB1 and EXO-WB2 reagents, store these buffers at +2/+8 °C;
- Upon arrival, store EXO-PK at +2/+8 °C;
- If properly stored, all the reagents provided with the kit are stable until the expiration date printed on the label.

Materials Required but Not Provided

- Protease inhibitor (Example: Sigma cat num. P8340)
- 96-100% Ethanol (Example: Sigma Aldrich, Cat. Num.02860) for dilution of EXO-WB1 and EXO-WB2 buffers and for DNA purification
- Disposable Gloves
- Single-use and/or pipettes with disposable tips
- Pipettes for reagent preparation
- Ultrapure water for dilution of 10X **EXO-IB**
- Heating block, or water bath for incubation at 56 °C
- Benchtop centrifuge with rotor for 2 ml reaction tubes (kit validated on Eppendorf 5415R) for Low Volume kit (EXO-SEL-LV).
- Vortex

Method Description and procedure

Method Description

Method: **SeleCTEV™ DNA** kit method is based on Exosomics' proprietary peptide-affinity method that selectively binds and enriches for tumor derived exosomes from which DNA is then extracted. **SeleCTEV™ DNA** affinity method also recovers circulating nucleic acids, making it the method of choice to recover the total circulating nucleic acid plus the tumor derived fraction from exosomes.

Sample type: Plasma 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.

Sample Volume: Each test requires at least 500 µl of plasma. Volumes can be scaled up to 2 ml (**SeleCTEV™ Low Volume**, cat.n.EXO-SEL-LV) or up to 7 ml (**SeleCTEV™ High Volume**, cat.n. EXO-SEL-HV), according to sample availability.

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

Special directions for the use of the Low Volume kit only are indicated by the product code "EXO-SEL-LV".

Procedure

SeleCTEV™ DNA allows the isolation of extracellular vesicles and tumor originated exosomes from plasma, and extraction of their DNA through a four-step procedure:

1. Reagent preparation
2. Plasma preparation
3. Cell free DNA and EV isolation from plasma
4. DNA purification

1 Reagent preparation:

1.2 **Isolation Agent (EXO-IA):** add 1 ml 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.

1.3 **1X Isolation Buffer (1X-IB):** dilute **10X Isolation Buffer (EXO-IB)** in fresh ultrapure water to a final 1X concentration (i.e. 1 ml of EXO-IB and 9 ml of ultrapure water) and label the vial as "1X-IB".

1.4 **Washing Buffer 1 (EXO-WB1):** add 9.4 ml of pure Ethanol (96-100%) to EXO-WB1 bottle (15 ml). Mix well by inverting 6-8 times.

1.5 **Washing Buffer 2 (EXO-WB2):** add 10.5 ml of pure Ethanol (96-100%) to EXO-WB2 bottle (15 ml). Mix well by inverting 6-8 times.

2 Plasma preparation:

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

2.2 Discard the pellet and debris and transfer the supernatant in the appropriate tube (EXO-IsoT-2 ml, 2 ml volume tubes for isolation with **SeleCTEV™ Low Volume)**

2.3 Dilute pre-cleared plasma with **1X-IB** buffer to a final 1:1 dilution (i.e. If processing 0.5 ml of plasma, add 0.5 ml of 1X-IB). If sample volume is >1 ml, split the sample into two isolation tubes (EXO-IsoT-2 ml) and then proceed to a final 1:1 dilution with **1X-IB** (i.e. If processing 2 ml of plasma, split sample equally in two isolation tubes and then add 1 ml of 1X- IB to each tubes).

2.4 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.)

3 Cell-free DNA and EV isolation from plasma:

3.1. Add resuspended **Isolation agent (EXO-IA)** to each vial of pre-cleared diluted sample.

EXO-SEL-LV: add 20 µl of **EXO-IA** to pre-cleared diluted plasma

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 16 000 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 1 ml of **1X Isolation Buffer (1X-IB)** directly on the pellet, without disrupting it. Spin the sample at 7000 g for 7 min at RT.

ⓘ If the pellet is not visible at this step, refer to Technical Support (Refer to Troubleshooting, p.6).

3.7. Repeat steps 3.5-3.6 one more time.

3.8. Discard the supernatant, carefully avoiding to dislodge the pellet. Eliminate the remaining supernatant from the tube with a pipette.

3.9. Resuspend (each) pellet in 200 µl of **Isolation Buffer (1X-IB)**.

ⓘ 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 (20 mg/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

If processing >1 ml of plasma, add 200 µl of EXO-LB to each isolation tube (EXO-IsoT-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 10 000 g for 1 min.

If processing >1 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 10 000 g for 1 min and discard the flow-through.

4.2.5 Add 500 µl of **Washing Buffer 2 (EXO-WB2)**, centrifuge at 10 000 g for 1 min and discard the flow-through.

4.2.6 Centrifuge 2 additional min at 16 000 g.

4.2.7 Transfer the spin column to an **Elution Tube (EXO-ColT-1.5 ml)**.

4.2.8 Elute the DNA from the column adding 20 µl of **Elution Buffer (EXO-EB)**.

4.2.9 Incubate for 5 min at RT.

4.2.10 Centrifuge 1 min at 14 000 g.

4.2.11 Transfer the eluate to the spin column, incubate for 5 minutes.

4.2.12 Repeat point 4.2.10 one more time. Discard the column.

4.2.13 Samples can now be used for further analyses or stored at -20 °C (EV-DNA can be stored directly in the EXO-ColT-1.5ml collection tube).

Troubleshooting

This table may solve some technical problems that could arise during **SeleCTEV™ DNA** protocol execution. For more information, please contact us at support@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 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

Poor DNA recovery		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 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.

Warnings and Precautions

SeleCTEV™ EV-DNA Enrichment kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay. To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.

All products sold by Exosomics are subject to extensive quality control procedures and are warranted to perform as described and used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. **Lysis Buffer (EXO-LB)** and **Washing Buffer 1 (EXO-WB1)** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

For more details, please refer to the product **SeleCTEV™ EV-DNA** Enrichment kit Safety Data Sheet.

General precautions

Always operate in accordance with Good Laboratory Practice (GLP) guidelines and the instructions included in this Handbook.

Handle and dispose of waste materials and reagents from the use of this kit in accordance with current regulations on staff safety and environmental protection.

Limitations

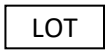
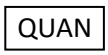
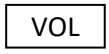
Results from the product must be interpreted within the context of all relevant clinical and laboratory findings and are not to be used alone for diagnosis.

The product is to be used only by professional users such as technicians, physicians and biologists experienced and trained in molecular biology techniques, including DNA isolation.

Dilution of the reagents, other than as described in this handbook, is not recommended and will result in a loss of performance.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

Symbols

	Contains reagents sufficient for n tests
	Catalogue number
	Batch or lot code
	Quantity
	Volume
	Use by
	Temperature limit
	Operating instructions
	CE Mark
	Manufacturer

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