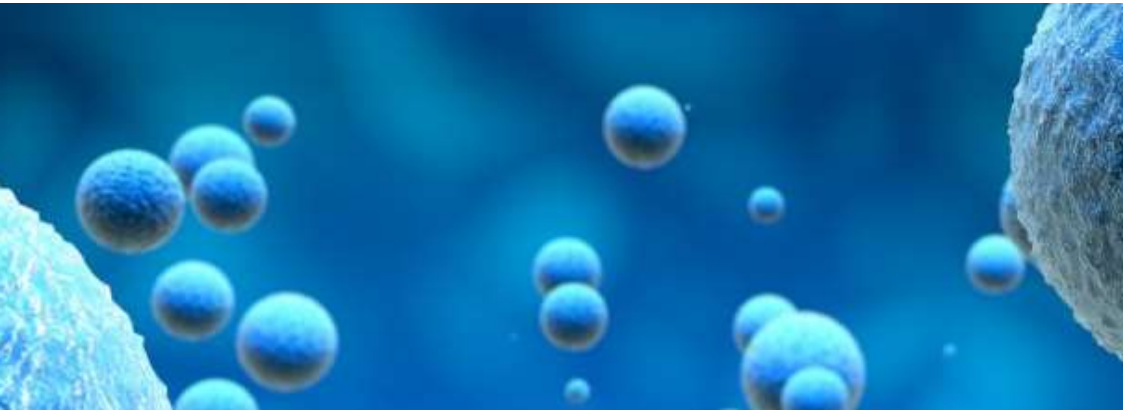

SoRTEV EV-RNA Enrichment kit

Low Volume

Cat.N. EXO-SOR-LV

www.exosomics.it

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1. PRODUCT OVERVIEW

EXOSOMICS SoRTEV™ Enrichment kit is an innovative workflow to selectively isolate tumor derived RNA (EV-RNA) contained in tumor enriched exosomes from biofluids. The purification is based on immune-affinity beads, coated with proprietary antibodies against exosome surface antigens and does not require any special equipment, such as ultracentrifugation or chromatography.

The **SoRTEV™ EV-RNA (EXO-SOR-LV)** yield the highest level of enrichment of tumor genetic material.

- **RNA extraction package** is designed by Exosomics experts to isolate EV-RNA from plasma and/or serum of the patient with a user-friendly protocol for RNA purification.

KIT SPECIFICATIONS

SoRTEV™	Cat. number	Volume	Biofluid	Extraction kit
Low Volume-RNA	EXO-SOR-LV	0.5 ml -2 ml	Plasma, serum	EV-RNA

FEATURES AND BENEFITS	
Unique	This kit is designed to selectively purify tumor-originated nucleic acids from tumor enriched exosomes from biofluids.
Fast and Accurate	No time-consuming ultracentrifugation step needed, turnaround time is minimum of 3 hours.
Versatile	Users can choose from different biofluid, input volumes (ranging from 0.5 ml up to 2 ml) and RNA-specific workflows.

2. PRINCIPLES OF THE PROCEDURE

The SoRTEV™ 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 exosomes, from a minimum of 500 µl of plasma/serum following two subsequent working steps as depicted below:

- EV-associated RNA isolation from biofluid of patient.
- RNA purification.

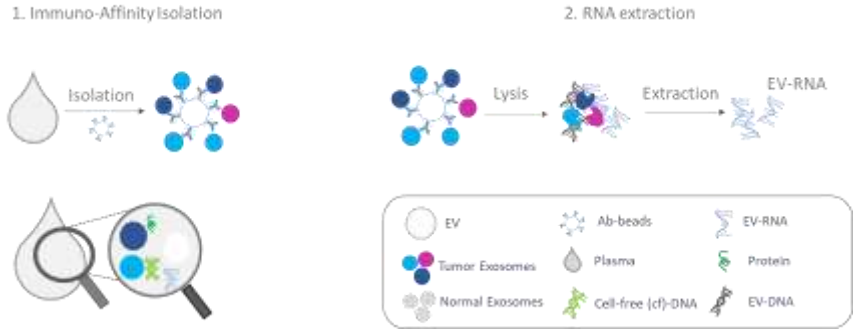

Figure 1 SoRTEV™ Isolation and EV-RNA Extraction kit

3. KIT COMPONENTS AND STORAGE

3.1 Kit Components:

Component	Name	Description	Amount	Storage
Isolation Agent	EXO-IA	Reagent for Isolation	1 vial (60 µl)	4°C
10X Isolation Buffer	EXO-IB	Diluent for Isolation	1 bottle (15 ml)	RT
10X Bead Wash Buffer	EXO-BW	Solution for bead Washing	1 bottle (15 ml)	4°C
Isolation Tubes	EXO-IsoT-2ml	Tubes for EV Isolation	48 tubes (2 ml)	RT
Lysis Buffer	EXO-LB	Solution for vesicle Lysis	1 bottles (30 ml)	4°C

Washing Buffer	EXO-WB	Solution for column Washing	1 bottles (30 ml)	4°C
Elution Buffer	EXO-EB	Solution for RNA Elution	1 bottle (4 ml)	RT
RNA purification columns	EXO-RC	Columns for RNA purification	24 Columns	RT
Elution Tubes	EXO-ColT-1.5ml	Tubes for pure RNA collection	24 Elution tubes (1.5ml)	RT

Customer supplied reagents and equipment:

- Protease inhibitor (Sigma cat num. P8340)
- RNase-free 2 ml tubes for Molecular Biology (24 tubes)
- Ethanol 96-100%
- Chloroform
- Disposable Gloves
- Single-use and/or pipettes with disposable tips
- Pipettes for reagent preparation
- MilliQ water
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Vortex

3.2 Storage Conditions: The SoRTEV™ 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 RNA EXTRACTION

Each test requires at least 500 µl of plasma or serum. Volumes can be scaled up to 2 ml, according to sample availability.

Sample Volumes: SoRTEV™ 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 1X-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 **Washing Buffer (WB):** add 20,9 ml of pure Ethanol (96-100%) in EXO-WB bottle (30 ml). Mix well by inverting 6-8 times.

2.2 **1X Bead Wash Buffer (1X-BW)**: dilute 10X Bead Wash Buffer (EXO-BW) in fresh milliQ water to a final 1X concentration (i.e. 1 ml of EXO-BW and 9 ml of mQ water) and label the vial as "1X-BW".

3 EV isolation from plasma or serum:

3.1. Add 2.5 µl of antibody-coated beads reagent (EXO-IA) to the pre-cleared diluted sample. If processing 2 ml of plasma/serum, repeat procedure from 3.1 to 3.7 using two (2) isolation tubes (EXO-IsoT-2ml).

3.2. Mix well by pipetting and inverting the tube.

3.3. Incubation time is 2 hours at RT under rotation.

3.4. Centrifuge 10 minutes at 9300g at RT.

3.5. Discard the supernatant and resuspend the pellet by gently adding 1ml of 1X-BW.

3.6. Spin the sample at 9300g for 10 min at RT.

3.7. Repeat steps **3.5-3.6** one more time**.**

Note: If processing 2 ml of plasma/serum, pool the pellets in one vial at this stage (3.6) and then proceed to section 4.

4 RNA purification:

4.1 EV Lysis:

4.1.1 Add 700 µl of lysis Buffer (EXO-LB) and vortex 30 seconds.
4.1.2 Incubate 5 minutes at RT.

Note: At this stage it is possible to freeze the sample at -80°C.

4.1.3 Add 140 µl of pure chloroform (not provided with the kit).
4.1.4 Shake the tube for 30 seconds.
4.1.5 Incubate 10 minutes at RT.
4.1.6 Incubate 1 minute in ice and centrifuge at 12000g at 4°C for 10 minutes
4.1.7 Transfer the top phase in a fresh tube (RNase-free 2 ml tube, not provided with the kit).
4.1.8 Add ethanol (96-100%) to the recovered phase in a 2:1 v/v ratio (i.e. add 900 µl of ethanol to 450 µl of recovered phase). Mix gently inverting 4-5 times.

4.2 RNA purification:

4.2.1 Transfer the mixture into a spin column (EXO-RC).
4.2.2 Spin at 14000g for 30 seconds.
4.2.3 Discard the flow through.
4.2.4 Repeat with the reminder.
4.2.5 Add 400 µl of RNA Washing Buffer (EXO-WB) in the spin column (EXO-RC).
4.2.6 Spin at 14000g for 30 seconds.
4.2.7 Discard the flow through.
4.2.8 Repeat steps **4.2.5 - 4.2.7** two more times.

4.2.9 Spin 2 additional minutes at 14000g to eliminate ethanol residues from the column.
4.2.10 Discard the flow through
4.2.11 Remove the tube and transfer the spin column into an elution tube (EXO-ColT-1,5ml).
4.2.12 Elute the column with 15 µl of elution buffer (EXO-EB).
4.2.13 Incubate 5 minutes at RT.
4.2.14 Spin 2 minutes at 200g. Spin 1 minute at 14000g, keep the flow through.
4.2.15 Eluted RNA is now ready for downstream analysis or for storage at -80°C.

Note: For low abundant targets, we advise to proceed immediately to downstream analysis to avoid RNA degradation during freeze-thawing cycles.

6. RELATED PRODUCTS

Exosomics Related Products

Kit	Cat. Number	Volume	Biofluid	Extraction kit
SeleCTEV™ Low Volume-Enrichment kit	EXO-SEL-LV	0.5 ml - 2 ml	Plasma, serum	cfDNA and EV-DNA
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 SoRTEV protocol execution. For more information, please contact us at info@exosomics.eu.

Technical Problems	Potential Causes	Suggestions and comments
Low RNA 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.
	Wash buffer (EXO-WB) prepared incorrectly	Check that these buffers were diluted in the correct volume of 96-100% ethanol (see page 6).
	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.

RNA not suitable for enzymatic reaction	Presence of ethanol traces in eluate	Make sure to remove all ethanol residuals from the column (EXO-RC) before eluting the sample.
	RNA degradation	Avoid RNA freeze-thawing cycles and keep it on ice while working. For long term storage, keep it at -80°C.

SoRTEV-RNA

Low Volume

Cat.N: EXO-SOR-LV

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