SoRTEV™ RNA Low Volume Enrichment Kit

User Manual – Version 2019/06

For Research Use Only

Σ 24 reactions

REF EXO-SOR-LV

EXOSOMICS SpA
Address: Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy
Tel: +39 0577 381308/ Fax +39 0577 381452
Business Registry: SI-137798 VAT#: IT01317090528
info@exosomics.eu www.exosomics.eu

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Intended Use

SoRTEV™ RNA Enrichment kit is a pre-analytical kit to isolate RNA contained in tumor enriched exosomes from plasma.
The **SoRTEV™ RNA Low Volume** (EXO-SOR-LVR) 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 RNA isolated with SoRTEV, followed by signal detection or amplification (PCR, real time PCR and digital PCR).

Exosomics **SoRTEV™ RNA** 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 assays.

Product Description

SoRTEV™ RNA Enrichment kit is ready-to-use and it is meant for running 24 tests. Kit allows the isolation of nucleic acids from tumor enriched exosomes, from a minimum of 500 µl of plasma following two subsequent working steps:

1. Exosome-associated RNA isolation from biofluid.
2. RNA purification.

The purification is based on Exosomics proprietary affinity method and does not require any special equipment, such as ultracentrifugation or chromatography, with a turnaround time of a minimum of 3 hours.

Materials Provided/Required

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

Kit Components

Product Code			**EXO-SOR-LV (0.5-2ml)**
Components	**Name**	**Description**	**Quantity (number/volume)**
Isolation Agent	EXO-IA	Reagent for isolation	1 vial (100 µl)
10X Isolation Buffer	EXO-IB	Diluent for isolation	1 bottle (15 ml)
10X Bead Wash Buffer	EXO-BW	Buffer for bead washing	1 bottle (15 ml)
Isolation Tubes	EXO-IsoT-2ml	Tubes for EV isolation	48 tubes (2 ml)
Lysis Buffer	EXO-LB	Buffer for vesicle lysis	1 bottle (30 ml)
Washing Buffer	EXO-WB	Buffer for column washing	1 bottle (30 ml)
Elution Buffer	EXO-EB	Buffer for RNA elution	1 bottle (4 ml)
RNA purification columns	EXO-RC	Columns for RNA purification	24 columns
Elution Tubes	EXO-ColT-1.5ml	Tubes for pure RNA collection	24 collection tubes (1.5 ml)

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

- Upon arrival store EXO-IA, EXO-BW and EXO-LB bottles at +2/+8 °C;
- Upon preparation of EXO-WB reagent, store buffer at +2/+8 °C;
- If properly stored, all the reagents provided with the kit are stable until the expiration date printed on the labels.

Materials Required but Not Provided

- ☐ Protease inhibitor (Example: Sigma Aldrich Cat num. P8340)
- ☐ 96-100% Ethanol (Example: RNAase-free Ethanol Sigma Aldrich, Cat. Num. 51976) for dilution of EXO-WB buffer
- ☐ Chloroform (Example: Sigma Aldrich Cat. Num. C2432)
- ☐ RNAse-free 2 ml tubes for Molecular Biology (24 tubes, Example: Starlab Cat. Num. S1620-2700)
- ☐ Disposable Gloves
- ☐ Single-use and/or pipettes with disposable tips
- ☐ Pipettes for reagent preparation
- ☐ Ultrapure water for dilution of (10X) **EXO-IB** and (10X) **EXO-BW**
- ☐ Benchtop centrifuge with rotor for 2 ml reaction tubes (kit validated on Eppendorf 5415R)
- ☐ Rotator or Shaker
- ☐ Vortex

Method Description and Procedure

Method Description

Method: **SoRTEV™ RNA** kit method is based on Exosomics' proprietary immuno-affinity method that enriches for tumor derived exosomes from which EV-RNA is then extracted. **SoRTEV™ RNA** affinity method yields the highest level of enrichment of tumor genetic material.

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: **SoRTEV™ RNA** kit 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 >1 ml of plasma, the best performance is obtained by splitting plasma into two 2 ml vials (EXO-IsoT-2ml) and then proceeding through steps 1-4 as for 1 ml samples, as described in Table 1.

Procedure

SoRTEV™ RNA allows the isolation of tumor originated exosomes from plasma, and extraction of their RNA through a four-step procedure:

1. Reagent preparation
2. Plasma preparation
3. EV isolation from plasma
4. EV-RNA purification

1 Reagent preparation:

1.1 **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".

1.2 **Washing Buffer (WB):** add 20.9 ml of pure Ethanol (96-100%) in EXO-WB bottle. Mix well by inverting 6-8 times.

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

2 Plasma preparation:

2.1 Pre-clear the 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-2ml, 2 ml volume tubes for isolation).

2.3 Dilute pre-cleared plasma in 1:1 v/v with 1X-IB (i.e. If processing 0.5 ml of plasma, add 0.5 ml of 1X-IB). If processing > 1ml of plasma, split the sample equally into two 2 ml isolation tubes (EXO-IsoT-2ml, included in the kit). Dilute pre-cleared plasma with 1X-IB to a final 1:1 v/v dilution. Please refer to Table 1 for plasma preparation.

2.4 Add protease inhibitor cocktail to each sample (1:1000 v/v protease: diluted plasma. Not provided with the kit).

Plasma Volume (ml)	1X-IB to be added (ml)	Total sample volume (ml)	Quantity of EXO-IsoT-2ml tubes	EXO-IA to be added (µl)
0.5	0.5	1	1	2
0.8	0.8	1.6	1	2
1	1	2	1	2
1.5	1.5	3	2	2 + 2
2	2	4	2	2 + 2

Table 1: Plasma processing and EV isolation. The table summarizes how to proceed to perform plasma dilution, how many isolation tubes (EXO-IsoT-2ml) to use, and the volume of isolation agent (EXO-IA) to be added per reaction.

3 EV isolation from plasma:

3.1. Add 2.0 µl of antibody-coated beads reagent (EXO-IA) to the pre-cleared diluted sample.

If processing >1 ml of plasma: add 2.0 µl of antibody-coated beads reagent (EXO-IA) into each (2) isolation tube (EXO-IsoT-2ml) and follow the procedure from 3.1 to 3.7 using two tubes. Refer to table 1 if processing other plasma volumes.

3.2. Mix well by pipetting and inverting the tube/s.

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

If a rotator is not available, a shaker can be employed for sample mixing during incubation.

3.4. Centrifuge 10 minutes at 9300 g at RT.

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

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

3.7. Repeat steps 3.5-3.6 one more time.

3.8. Discard the supernatant.

If processing >1 ml of plasma, pool the pellets in one vial at this stage (3.6), i.e. Resuspend pellet 1 with 1 ml of 1X-BW and transfer the resuspended pellet into the vial containing pellet 2. Resuspend the second pellet. You now have pooled the two pellets in the same vial, proceed to step 3.8 prior to EV Lysis (4.1).

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.

ⓘ 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 12000 g 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 at RT.

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 14000 g for 30 seconds at RT.

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 14000 g at RT 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-1.5 ml).

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 200 g at RT. Spin 1 minute at 14000 g at RT, keep the flow through.

4.2.15 Eluted RNA is now ready for downstream analysis or for storage at -80 °C.

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

Trouble-shooting

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

Low RNA recovery	Prolonged sample storage at room temperature	Do not keep the samples at RT for prolonged time.
	Extraction efficiency	To track RNA extraction efficiency, we advise to use a synthetic RNA template (Example: Tataa Biocenter, Cat. Num. RS25PFI), which can be added after step 4.1.2 (EV Lysis).
	Wash buffer (EXO-WB) prepared incorrectly	Check that these buffers were diluted in the correct volume of 96-100% ethanol.
	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.
Presence of DNA traces in RNA eluate	DNA carry over during RNA purification	We suggest to perform DNase treatment (Example: Sigma-Aldrich, Cat. Num. AMPD1-1KT) on beads/EVs pellet (step 3.7). If using Sigma-Aldrich Amplification Grade DNase I, incubate sample with DNase I and 1X Reaction Buffer for 10 min at RT. The reaction does not require inactivation, only addition of lysis buffer is required.

Warnings and Precautions

SoRTEV™ RNA 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 assay. To minimize irregularities in 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**) contains phenol and guanidinium salts, and should be handled with care. Phenol should be handled under chemical hood. 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 **SoRTEV™ RNA** Safety Data Sheet.

General Precautions

Always operate in accordance with good laboratory 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 biological techniques, including RNA 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
REF	Catalogue number
LOT	Batch or lot code
	Use by
	Temperature limit
	Operating instructions
	Manufacturer

Contact Information

Exosomics S.p.A.
Address: Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy
Business Registry: SI-137798 **VAT#:** IT01317090528
Administration:
Tel: +39 0577 381308
For info: administration@exosomics.eu
Technical Support:
For info: support@exosomics.eu
Sales & Marketing:
Tel: +39 0577 381309