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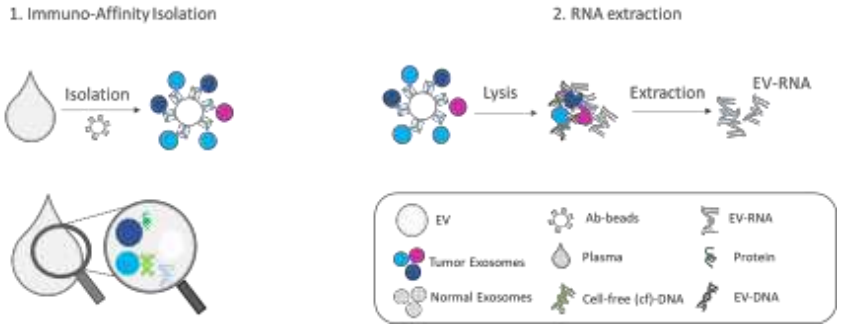
*LiqBiopsy Tumor Exosome RNA Kit

(Cat# K1254-24; 24 Reactions; Store at 4°C), *Not for sale in Italy and Austria*

I. Introduction:

LiqBiopsy Tumor Exosome RNA Kit is an innovative workflow to selectively isolate tumor derived RNA (EV-RNA) contained in tumor 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 LiqBiopsy Tumor Exosome RNA Kit yields the highest level of enrichment of tumor genetic material. It doesn't require the time-consuming ultracentrifugation steps. The turn-around time is a minimum of 3 hours. Additionally, users can choose from different biofluids including serum, plasma, input volumes (ranging from 0.5 ml up to 2 ml) and RNA- specific workflows.



II. Key Features:

- Ready-to-use
- Selectively purifies tumor- originated nucleic acids from tumor enriched exosomes from biofluids.
- RNA purification, volume. 0.5 ml-2 ml
- No time-consuming ultracentrifugation step needed
- Turnaround time is a minimum of 3 hours.
- Users can choose from different biofluids, input volumes (ranging from 0.5 ml up to 2 ml) and RNA- specific workflows.

III. Application:

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

IV. Kit Contents:

Kit Components	K1254-24	Part Number	Storage Temperature
Isolation Agent	1 vial (60 µl)	K1254-24-1	4°C
10X Isolation Buffer	1 bottle (15 ml)	K1254-24-2	RT
10X Bead Buffer	1 bottle (15 ml)	K1254-24-3	4°C
Isolation Tubes	48 tubes (2 ml)	K1254-24-4	RT
Lysis Buffer	1 bottles (30 ml)	K1254-24-5	4°C
Washing Buffer	1 bottles (30 ml)	K1254-24-6	4°C
Elution Buffer	1 bottle (4 ml)	K1254-24-7	RT
RNA purification columns	24 Columns	K1254-24-8	RT
Elution Tubes	24 Elution tubes (1.5 ml)	K1254-24-9	RT

V. User Supplied Reagents and Equipment:

- Protease inhibitor
- 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

VI. Shipment and Storage:

All the reagents are shipped at controlled temperature (4-8°C) with ice packs. All components must be stored carefully, according to the protocol. Properly sealed reagents are stable at the indicated storage temperature for at least 12 months after kit delivery.

VII. Handling of blood and plasma:

Blood and plasma should be handled with care. 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.

VIII. 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: LiqBiopsy Tumor Exosome RNA Kit 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 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 (Isolation Tubes-2ml).
1.3 Dilute 10X Isolation Buffer in fresh milliQ water to a final 1X concentration (i.e. 1 ml of Isolation Buffer and 9 ml of mQ water) and label the vial as "1X- Isolation Buffer". Dilute pre-cleared plasma or serum in 1:1 v/v with 1X- Isolation Buffer (i.e. If used 0.5 ml of plasma, add 0.5 ml of 1X Isolation Buffer). If processing 2 ml of plasma/serum, split the sample into two 2 ml isolation tubes, 2ml and dilute 1 ml of pre-cleared plasma with 1 ml of 1X- Isolation Buffer.
1.4 Add protease inhibitor cocktail to each sample (1:1000 v/v protease: diluted plasma). *Not provided with the kit.*

2 **Reagent preparation:**
2.1 **Washing Buffer:** add 20,9 ml of pure Ethanol (96-100%) in Washing Buffer (30 ml). Mix well by inverting 6-8 times.
2.2 **1X Bead Wash Buffer**: dilute 10X Bead Wash Buffer in fresh milliQ water to a final 1X concentration (i.e. 1 ml of Bead Wash Buffer and 9 ml of mQ water) and label the vial as "1X Bead Wash Buffer".

3 **EV isolation from plasma or serum:**
3.1. Add 2.5 µl of antibody-coated beads reagent (Isolation Agent) to the pre-cleared diluted sample. If processing 2 ml of plasma/serum, repeat procedure from 3.1 to 3.7 using two isolation tubes, 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 Bead Wash Buffer.
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 (Lysis Buffer) 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 RNA Purification Column.
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 in the RNA Purification Columns.
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 (1.5ml).
4.2.12 Elute the column with 15 µl of elution buffer.
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.

IX. General Troubleshooting Guide:

Problems	Potential Causes	Solution
Low RNA recovery	. Poor plasma quality due to delayed blood processing. Repeat blood processing . Plasma samples are frozen and thawed multiple times . Prolonged sample storage at room temperature . Wash buffer (EXO- WB) prepared incorrectly . The eluate volume is lower than the applied volume	. Always use fresh samples or samples thawed once. . Do not keep the samples at RT for prolonged time. . Check that these buffers were diluted in the correct volume of 96-100% ethanol . 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 . RNA degradation	. Make sure to remove all ethanol residuals from the column before eluting the sample. . Avoid RNA freeze-thawing cycles and keep it on ice while working. For long term storage, keep it at -80°C.

FOR RESEARCH USE ONLY! Not to be used on humans.