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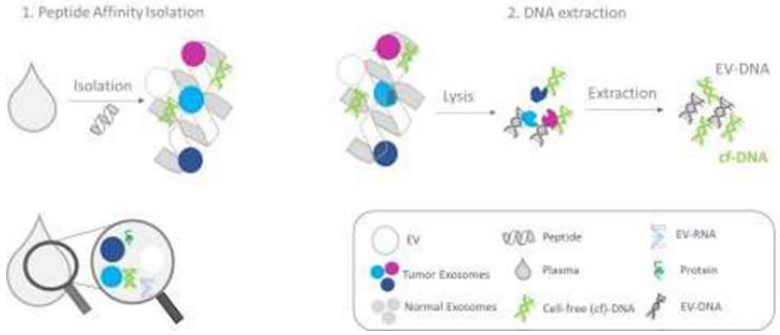
*LiqBiopsy Tumor Exosome DNA Kit II

(Cat# K1256-20; 20 Reactions; Store at 4°C), *Not for sale in Italy and Austria*

I. Introduction:

LiqBiopsy Tumor Exosome DNA Kit II 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. LiqBiopsy Tumor Exosome DNA Kit II kit is versatile: users can choose from different biofluid input volumes (ranging from 2-3 ml up to 7 ml) and DNA-specific workflows.



II. Key Features:

- User-friendly protocol
- **Unique**: 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 a minimum of 4 hours.
- **Versatile:** Users can choose from different biofluids, input volumes (ranging from >2 ml up to 7 ml) and DNA- specific workflows.

III. Application:

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

IV. Kit Contents:

Components	K1256-20	Part Number	Storage Temperature
Isolation Agent	1 vial (2 mg)	K1256-20-1	4°C
Resuspension Buffer	1 vial (1 ml)	K1256-20-2	4°C
10X Isolation Buffer	1 bottle (30 ml)	K1256-20-3	RT
Isolation Tubes	20 tubes (15 ml)	K1256-20-4	RT
Proteinase K	1 ml (20 mg/ml)	K1256-20-5	4°C
Lysis Buffer	1 bottle (4 ml)	K1256-20-6	RT
Washing Buffer 1	1 bottle (15 ml)	K1256-20-7	4°C
Washing Buffer 2	1 bottle (15 ml)	K1256-20-8	4°C
Elution Buffer	1 bottle (4 ml)	K1256-20-9	RT
DNA Purification Columns	20 Columns	K1256-20-10	RT
Elution Tubes	20 Elution tubes (1.5 ml)	K1256-20-11	RT

V. User Supplied Reagents and Equipment:

- Protease inhibitor
- 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 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. Exosome isolation and DNA extraction

Each test requires at least 500 µl of plasma or serum. Volumes can be scaled up to 7 ml, according to sample availability.
Sample Volumes: LiqBiopsy Tumor Exosome DNA Kit II has been optimized for sample volumes ranging from 2-3 ml to 7 ml of plasma or serum.

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 **Isolation Agent:** add 800µl of Resuspension Buffer into the Isolation agent 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:** add 9.4 ml of pure Ethanol (96-100%) in Washing Buffer 1 (15 ml). Mix well by inverting 6-8 times.

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

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

3.1. Add 40µl of resuspended Isolation agent to the 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 1 ml of 1X Isolation buffer directly on the pellet without disrupting it. Spin the sample at 7000 g for 7 min at RT.

3.7. Repeat steps 3.5-3.6 one more time.

3.8. Resuspend the pellet in 200 µl of Isolation Buffer. *Note: we advise to proceed directly to step 4 (DNA purification) for maximal DNA recovery.*

4 DNA purification:

4.1 **EV Lysis:**

4.1.1 Add 20 µl of Proteinase K (20mg/ml)

4.1.2 Add 200 µl of Lysis Buffer

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% and mix by briefly vortexing the tube.

4.2.2 Transfer the mixture in a DNA Spin Column and centrifuge at 10000 g for 1 min. Discard the flow-through.

4.2.3 Add 500 µl of Washing Buffer 1, centrifuge at 10000 g for 1 min and discard the flow-through.

4.2.4 Add 500 µl of Washing Buffer 2, centrifuge at 10000 g for 1 min and discard the flow-through.

4.2.5 Centrifuge 2 additional min at 16000 g.

4.2.6 Transfer the spin column to an Elution Tube.

4.2.7 Elute the DNA from the column adding 50 µl of Elution Buffer.

4.2.8 Incubate for 5 min at RT.

4.2.9 Centrifuge 1 min at 14000 g. Samples can now be used for further analyses or stored at -20°C

VIII. General Troubleshooting Guide:

Technical Problems	Potential Cause	Solution
Poor DNA recovery	. Use of anticoagulants other than EDTA may not fully preserve circulating DNA. . Repeat blood collection . Poor plasma quality due to delayed blood processing. Repeat blood processing to plasma. . Prolonged sample storage at room temperature . Plasma samples are frozen and thawed multiple times . Prolonged sample storage at room temperature . Incomplete resuspension of the peptide . No visible pellet . Lysis buffer and pellet-proteinase K solutions not sufficiently mixed . Inefficient sample lysis . Sub-optimal ethanol percentage . Clogged DNA spin column . Wash buffers 1 and 2 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. . Peptide solution may initially look cloudy after resuspension in resuspension buffer. Do not vortex the solution, simply tap the vial to resuspend the peptide. Make sure that the peptide is fully resuspended in resuspension buffer and the final solution looks clear. . It may occasionally occur but should not affect DNA recovery. . Mix lysis buffer and pellet-proteinase K solution well by pipetting up and down and vortexing at least 30'' to completely resuspend the peptide pellet. . Use fresh proteinase K. If needed, increase incubation time with proteinase K. . Use fresh 96-100% ethanol. Do not use denatured alcohol which may contain methanol. . Repeat the procedure increasing the incubation time in proteinase K. . Check that these buffers were diluted in the correct volume of 96-100% ethanol (see page 8). . 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 . Extremely low or no DNA recovered . Not optimized elution volume . New PCR assay . Interference due to plasma inhibitors	. Make sure to remove all ethanol residuals from the column (EXO-DC) before eluting the sample. . See poor DNA recovery section above for troubleshooting. . Calculate the optimal elution volume for PCR reaction. . If the PCR assay is changed, readjust the eluate volume. . 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.

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