

ELAEXIA®-T MS kit

1 Description

1.1 Kit components

One vial contains 200µL ELAEXIA®-T MS stock solution for 10 exosome enrichments from human plasma.

1.2 Capacity

20µL of ELAEXIA®-T MS stock solution are sufficient to label 1mL of plasma exosomes for flow cytometry sorting in a total reaction volume of 2mL.

1.3 Storage

Store protected from light at 2-8°C. Do not freeze. The expiration date is indicated on the vial label.

1.4 Background & principle

Exosomes are extracellular vesicles released from cells reflecting both the phenotype and functional state of their cell of origin and bear great promise to serve as biomarkers. ELAEXIA®-T MS has been developed for specific binding to exosomes from human plasma.

ELAEXIA®-T MS specifically binds to phosphatidylethanolamine of the outer leaflet of the exosome lipid bilayer membrane, which is ubiquitously present in all exosomes independent of their origin.

1.5 Application

Enrichment of ELAEXIA®-T MS-labelled exosomes from plasma by flow cytometry sorting.

The kit has been optimized for downstream proteomic analysis by mass spectrometry (MS).

1.6 Reagent, materials and instrument requirements

Reagents, Materials & Instruments

- S-monovettes EDTA, Butterfly syringes
- PBS pH 7.2
- Eppendorf-tubes, cryo-tubes
- Amicon Ultra Centrifugal Filter, 100kDa MWCO, 4ml
- 15mL ultracentrifugation tubes
- 20µm sterile syringe filters
- Parafilm, filter-paper
- Centrifuges (Table- & Eppendorf- & Ultra-centrifuge)
- End-over-end rotator
- Flow cytometer sorter

2 Protocol

2.1 Plasma preparation

- Draw blood using S-monovette EDTA and butterfly syringe (do not use evacuated tube systems!), transfer blood into suitable tube and centrifuge at 2,000xg at 4°C for 15min to separate plasma from cells
- Transfer supernatant to suitable tube and repeat centrifugation at 2,000xg at 4°C for 15min
- Transfer supernatant plasma to suitable tube and centrifuge at 10,000xg at 4°C for 15min to spin-down debris
- Use supernatant plasma for labelling or store aliquots at -80°C

2.2 Labelling exosomes with ELAEXIA®-T MS

- Centrifuge ELAEXIA®-T MS stock solution at 10,000xg at 4°C for 1min to remove aggregates
- Add 1mL PBS pH 7.2 to 980µL plasma and gently shake
- Add 20µL of ELAEXIA®-T MS stock solution
- Incubate the 2mL reaction volume for 60min at room temperature protected from light on the end-over-end rotator

2.3 Flow-cytometry sorting of labelled exosomes

- Filter labelled plasma sample into 15mL tube through 20µm-sterile filter
- Prepare 2x15mL tubes with 100µL PBS per sample to be sorted
- Perform sorting of sample into the 2x15mL tubes in purity mode (sample volume is pending number of events – expect 1-5mL total volume for sorted sample)

A-gain	0.6
H-gain	1.0
Voltage	400
Threshold (%)	0.6%
Trigger	488-SSC
Limit	None
Mode	Purify
Drop Envelope	1

Tab 1: Settings for the Beckman Coulter MoFlo Astrios^{EQ} instrument for sorting of labelled exosomes

2.4 Concentration of sorted exosomes

Sorted exosomes can be concentrated by centrifugation through Amicon-filter or by ultracentrifugation.

Amicon-centrifugation:

- Concentrate sorted samples down to 100µL using Amicon Ultra-100kDa filter according to manufacturer instructions

Ultra-centrifugation:

- Transfer sorted sample into ultracentrifugation tube, fill-up tube with PBS and ultracentrifuge at 100,000xg at 10°C for 90min.
- Remove supernatant and place tube upside down on filter-paper for 5min at room temperature to remove as much fluid as possible from the pelleted exosomes.
- Seal tube with Parafilm and store at -80°C until use for MS proteomic analysis

3 Example

Ultracentrifuged samples were lysed in 100µl of 0.1% n-dodecyl-beta-D-maltoside and 0.1M triethylammonium bicarbonate buffer, pH 8.5, and subsequently heat-inactivated (95°C for 10min, 800rpm). After determination of protein concentration by BCA assay, samples were reduced and alkylated with 2mM DL-dithiothreitol (37°C for 60min at 800rpm) and 2mM iodoacetamide (room temperature for 60min and 800rpm in the dark). Lysates were digested overnight (37°C, 16h, 800rpm) by adding 250ng of trypsin gold/rLysC mixture per replicate. Peptides were prepared for C18-stage tipping by diluting the sample volume 1:5 with loading buffer (10% acetonitrile, 3% trifluoroacetic acid), desalted with three layers C18 stage tips, and finally resuspended in 10µl of 0.1% formic acid. Analysis was performed by LC-MS (Bruker nano Elute 2-timsTOF Ultra 2).

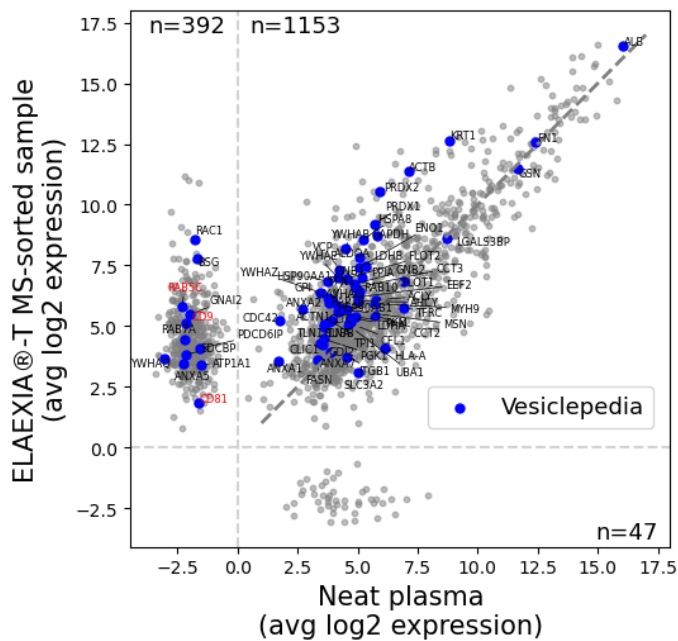


Fig 1: Average log2 protein abundance of ELAEXIA®-T MS-sorted samples against neat plasma measurements. Proteins found in the top 80 Vesiclepedia marker list are marked in blue/red.

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Further information

Refer to www.therawis.com for safety data sheets.

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