Instructions for Use

Research Use Only!

therawis diagnostics

ELAEXIA® FC kit

1 Description

1.1 Kit components

Vial 1 contains 10µL ELAEXIA® FC stock solution. Vial 2 contains 200µl buffer A.

Dilute 10µL ELAEXIA® FC with 190µL buffer A to obtain 200µL ELAEXIA® FC working solution for 40 exosome stainings from human plasma or cell culture supernatant.

1.2 Capacity

5µL ELAEXIA® FC working solution are sufficient to label 20µL exosomes from diluted plasma or undiluted cell culture supernatant for flow cytometry detection in a total reaction volume of 25µL.

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® FC has been developed for specific binding to exosomes from human plasma and cell culture supernatant.

ELAEXIA® FC specifically binds to phosphatidylethanolamine (PE) of the outer leaflet of the exosome lipid bilayer membrane, which is ubiquitously present in all exosomes independent of their origin.

1.5 Application

Detection of ELAEXIA® FC-labelled exosomes from plasma or cell-culture supernatant by flow-cytometry.

1.6 Reagent, materials and instrument requirements

Reagents, Materials & Instruments:

- S-monovettes EDTA, Butterfly syringes
- NaCl, 10% Triton X-100
- Cell culture medium with/without FCS
- Eppendorf-tubes, cryo-tubes
- Table & Eppendorf centrifuge
- Flow cytometer

2 Protocol

2.1 Sample volume calculation

- Calculate the number of samples to be investigated and prepare excess total volume to account for dead volumes
- Each reaction volume for staining is 25µL: 20µl sample (1:5 diluted plasma or undiluted cell culture supernatant) + 5µL ELAEXIA® FC working solution
- Account for two negative controls per sample: Triton X-100-control (to solubilize exosome membranes) and NaCl-control (to replace ELAEXIA® FC working solution)

2.2 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 supernatant plasma from cells
- Transfer supernatant plasma 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 staining or store aliquots at -80°C

2.3 Cell-culture supernatant preparation

- Culture cells to optimal confluency
- Incubate cells with cell culture medium without FCS for 16h
- Harvest supernatant, centrifuge at 2,000xg at 4°C for 15min
- Use supernatant for staining or store aliquots at -80°C

2.4 Staining with ELAEXIA® FC

- Centrifuge ELAEXIA® FC at 10,000xg at 4°C for 1min to remove aggregates
- Prepare 1:4 pre-diluted plasma: add 16µL NaCl to 4µL plasma and mix gently

Per 20µL sample (1:4 pre-diluted plasma or undiluted cell culture supernatant):

- Add 5µL ELAEXIA® FC working solution to sample, resuspend gently and incubate 25µL reaction volume 30min at room temperature protected from light and softly shake at 300rpm

2.5 Negative controls

- For Triton X-100 control: Add 2µL of 10%-Triton X-100 to 20µL sample (1:5 pre-diluted plasma or undiluted cell culture supernatant), incubate for 60min at room temperature and softly shake at 450rpm. Take 20µL for ELAEXIA® FC staining (see section 2.4)
- For NaCl control: replace 5µL ELAEXIA® FC by 5µL NaCl in 25µL reaction volume

2.6 Final dilution for measurement

- Serially dilute reaction volumes to final dilutions of 1:1000 (plasma) and 1:100 (cell culture supernatant) and use 200µL of final dilutions for flow-cytometry measurements

2.7 Counting beads (optional)

For quantification of events counting beads can be added.

2.8 Flow-cytometry measurement and analysis

FSC gain	11.00
SSC voltage (SC)	28.00
Threshold (%)	0.10
Flow rate	1.00
Mode	Normalized
PMT voltage (%)	65.00

Tab 1: Settings for the Sony SA3800 spectral cell analyzer for detection of labelled exosomes

3 Examples

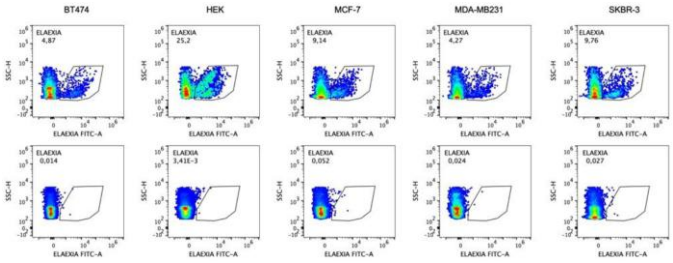

Fig 1: Cell culture supernatants from various cell lines were labelled with ELAEXIA® FC and analyzed by flow cytometry (upper panel). The lower panel shows the cell culture supernatants after solubilization of the exosome membranes with 1% Triton X-100 (negative control).

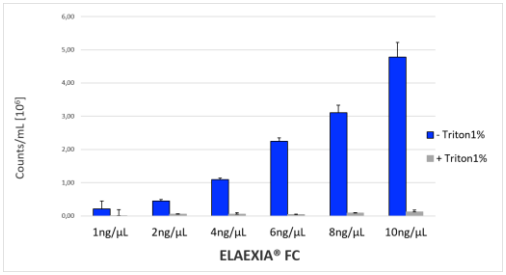

Fig 2: Concentration-dependent detection of ELAEXIA® FC-labelled exosomes from human plasma (blue bars). The same plasma samples were treated with Triton X-100 to solubilze the exosome membranes as negative control (grey bars).

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Further information

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