evGAG

Extracellular vesicles purification kit for plasma, serum, urine

User Manual – Version 2020/09

For Research Use Only (RUO)

Σ 20 reactions

REF EXO-EVG-20

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Intended Use

Exosomes are nano-sized (50-120 nm) vesicles released into surrounding body fluids by exocytosis by most living cells, under both normal and pathophysiological conditions. They have largely been recognized for their role in mediating intercellular communication by serving as carriers of different biomolecules, including proteins, lipids and genetic material. Thus, exosomes and their biologically active cargos may offer diagnostic and prognostic information in a range of diseases.

evGAG *Extracellular vesicles purification kit for plasma, serum, urine* is a pre-analytical kit to purify extracellular vesicles (EVs) and exosomes from plasma, serum and urine

evGAG *Extracellular vesicles purification kit for plasma, serum, urine (**EXO-EVG-20**)* kit is optimized for input volumes ranging from 0.5 ml.

Exosomics **evGAG** *Extracellular vesicles purification kit for plasma, serum, urine* kit does not provide a diagnostic result. It is responsibility of the user to use and validate the kit in conjunction with any downstream assay.

Product Description

evGAG *Extracellular vesicles purification kit for plasma, serum, urine* is a precipitation reagent intended for the purification of exosome and extracellular vesicles (EVs) from biological fluid samples (plasma, serum, urine). **evGAG** *Extracellular vesicles purification kit for plasma, serum, urine* is based on the ability of certain cationic agents to neutralize the negative charge derived from the presence of glycosaminoglycans (GAGs) on the surface of EVs, and to aggregate in the form of complexes that can be then purified by centrifugation. EVs are purified in pellet format, following an easy and no time-consuming protocol (20 minutes), and after resuspension in the appropriate buffer it allows downstream analyses of protein profiling (Western Blot, ELISA, Flow cytometry) and genetic profiling (mRNA, miRNA, NGS sequencing, ddPCR, Beaming).

List of kit components

Kit components, meant to run a total of 20 reactions.

Product Code (Input Volume)			**EXO-EVG-20 (2 X 10 ml)**
Components	**Name**	**Description**	**Quantity (volume/number)**
Precipitation Agent	evGAG	Liquid Reagent for EVs isolation	2 vials (10 ml)

Materials Required but Not Provided

- ☐ Disposable Gloves
- ☐ Single-use and/or pipettes with disposable tips
- ☐ Refrigerate microcentrifuge
- ☐ 1.5 ml microcentrifuge collection tubes

Storage and stability

The Kit is shipped at room temperature and the components should be stored at room temperature in the dark. Do not freeze.

Properly stored kit is stable until the expiry date stated on the vial label.

Method Description and procedure

Procedure

1 Sample preparation:

1.2 Collect the **plasma/serum/urine** sample in sterile tube and centrifuge at 2000 g for 5 minutes to remove cells and cellular debris. If additional debris remains detectable, centrifuge the supernatant for additional 10 minutes at 2000 g. If the sample has been frozen, thaw and temper it before processing.

1.3 Transfer the Supernatant to a new tube and discard the pellet.

2 EV isolation:

2.1 Vortex evGAG vial before use.

2.2 Dilute the **plasma/serum/urine** sample with evGAG to a final 1:2 v/v **refer to table 1.** (i.e. If processing 0.5 ml of plasma/serum/urine sample volume, add 1 ml of evGAG).

Sample	Sample Volume (ml)	evGAG Volume (ml)
Plasma/Serum/Urine	0.5	1
NTA (Nanoparticle Tracking Analysis)*	0.05	0.1
Flow cytometry analysis*	0.1	0.2
Other	Request	Request

Table 1. The table summarizes how to proceed to perform sample dilution. * For Nanoparticle Tracking Analysis (NTA) and Flow cytometry analysis proceed to specific protocol reported below, respectively **Protocol A** and **Protocol B**.

2.3 Mix well by inverting or vortexing the tube (the solution will have a characteristic blue color).

2.4 Incubate the sample for 5 minutes at 4°C. The tubes do not need to be rotated during the incubation.

2.5 Centrifuge the sample at 16000g for 15 minutes at 4°C.

2.6 Carefully remove the supernatant. The pellet will be dark blue, **refer to Figure 1.**

2.7 Resuspend the pellet in the appropriate buffer, depending on the downstream analysis, repeatedly pipetting up and down. Resuspended EVs can be used for analysis or stored at -80°C.

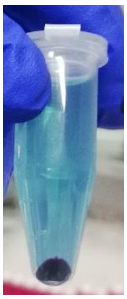

Figure 1. EVs pellet obtained from plasma sample, step 2.6 (example: 0.5 ml plasma sample with 1 ml evGAG)

Protocol A: Nanoparticle Tracking Analysis (NTA) Protocol

Protocol A:

1a. Collect plasma sample in a sterile tube and centrifuge at 2000 g for 5 minutes to remove cells and cellular debris. If additional debris remains detectable, centrifuge the supernatant for additional 10 minutes at 2000 g. (For the isolation of EVs by ultracentrifugation, 0.120 ml of plasma were used).

2a. Transfer the supernatant to a new tube and discard the pellet.

3a. Collect 0.05 ml of the plasma/serum/urine supernatant, add 0.1 ml of evGAG and follow the evGAG kit protocol (step **2.1-2.6**).

4a. Resuspend the pellet containing EVs in 1 ml Phosphate buffered saline (PBS) modified without calcium chloride and magnesium chloride.

5a**.** Proceed to NTA measures.

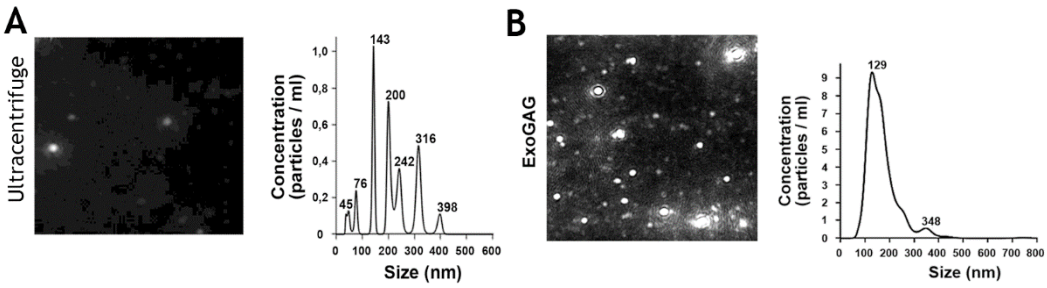

Figure 2. Nanoparticle tracking analysis (NTA) image of plasma isolated EVs showing particle size (nm) and concentration (particles/ml) comparing EVs isolated by (**A**) ultracentrifugation or by (**B**) evGAG.

Protocol B: Flow cytometry analysis

Protocol B:

1b. Collect plasma sample in a sterile tube and centrifuge at 2.000 g for 5 minutes to remove cells and cellular debris. If additional debris remains detectable, centrifuge the supernatant for additional 10 minutes at 2.000 g.

2b. Transfer the supernatant to a new tube and discard the pellet.

3b. Collect 0.1 ml of the plasma supernatant, add 0.2 ml of evGAG and follow the evGAG kit protocol (step 2.1-2.6).
4b. Resuspend the pellet containing EVs in 0.2 ml Phosphate buffered saline (PBS).
5b. Incubate the sample for 1 h with EVs antibodies detecting exosome markers at 4°C in rotation (example: Antibody anti-CD9, Santa Cruz Biotechnology, cat. Num. sc13118, dil. 1:50). Proceed to prepare the negative control, incubating it with only the secondary antibody.
6b. Wash the sample once with PBS by centrifugation at 16000 g for 15 minutes at 4°C.
7b. Add secondary antibody (example: anti -mouse Alexa488, Abcam, cat. Num. ab150113, dil. 1:1000) and incubate for 1 h at 4°C in rotation.
8b. Wash the sample once with PBS by centrifugation at 16000 g for 15 minutes at 4°C.
9b. Analyze by flow cytometry.

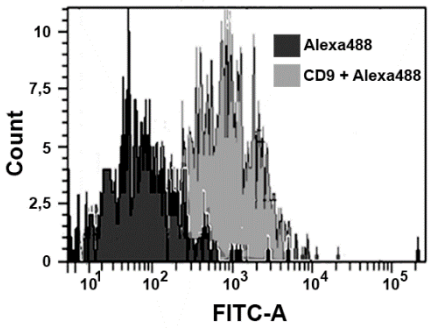

Figure 3. Examples of cytometry analysis of plasma EVs isolated using evGAG. FITC-A: Fluorescein isothiocyanate A.

Recommendations and warning

The purification kit is for research use only and for single use only and for professional use only.
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
The reagent contains acetic acid. Keep away from heat, hot surfaces, sparks, open flames and all other sources of ignition. Do not smoke. Wear protective gloves/garments/glasses/mask. In the case of contact with skin or eyes, rinse with plenty of water. Do not pipette by mouth. Avoid aerosols.
For more details, please refer to **evGAG** Safety Data Sheet (SDS).
Do not freeze the reagent and protect from light. Samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed. Do not use reagents after expiration date indicated on the vial.
Avoid microbial contamination of the reagent.
Any serious incident related to the product must be reported to the manufacturer.

Technical support

For more information, please contact our technical support at: support@exosomics.eu

Symbols

	Contains reagents sufficient for n tests
REF	Catalogue number
LOT	Batch or lot code
QUAN	Quantity
VOL	Volume
	Use by
	Temperature range
	Operating instructions
	Manufacturer

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