evGAG
Extracellular Vesicles purification kit

User Manual – Version 2020/09

For Research Use Only (RUO)

Σ 20 reactions

REF HBM-EXS-GAG

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Intended Use

Exosomes and Extracellular Vesicles (EVs) are nano-sized vesicles released into surrounding body fluids 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 EVs and their biologically active cargos may offer diagnostic and prognostic information in a range of diseases.

evGAG *Extracellular vesicles purification kit* is a pre-analytical kit to purify extracellular vesicles (EVs) from biological fluids.

evGAG *Extracellular vesicles purification 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* is a precipitation reagent intended for the purification of extracellular vesicles (EVs) from human or animal biofluids (plasma, urine, serum, CSF, bronchoalveolar lavage etc.).

How it works: **evGAG** is based on the ability of certain cationic agents to neutralize the negative charge of glycosaminoglycans (GAGs) on the surface of EVs and to aggregate in the form of complexes that can be separated by centrifugation. EVs are purified in pellet format, and after resuspension in the appropriate buffer (PBS 1x) are suitable for protein biomarker profiling (Western Blot, ELISA, Flow cytometry) or genetic profiling (mRNA, miRNA, NGS sequencing, ddPCR, Beaming). The turnaround time (TAT) for the protocol is 20 minutes.

List of kit components

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

Component	Quantity/Volume	Storage
evGAG (liquid)	2 x 10 ml bottles	Room temperature (RT)

Materials Required but Not Provided

- Disposable **PPE** *Personal Protection Equipment*
- Single-use and/or pipettes with disposable tips
- Refrigerate microcentrifuge
- 1.5 ml microcentrifuge collection tubes

Storage and stability

The Kit is shipped and must be stored at room temperature in the dark. Do not freeze.
Properly stored kit is stable until the expiry date stated on the product label.

Method Description and procedure

Procedure

1 Sample process:

1.1 process the samples in a shorter time possible after collection;

1.2 aliquoting is recommended since freeze-and-thaw cycles reduce the quality of the sample;

1.3 store the biofluid aliquots at -80°C or proceed with **evGAG** *Extracellular vesicles purification kit* protocol;

1.4 If working with frozen aliquots of urine, thaw them at RT, then warm the sample at 37°C for 15 in a water bath.

2 Starting volume and sample preparation

Recommended starting volumes of biofluids:

Sample	Volume recommended
Plasma	0.5 ml – 2 ml
Serum	0.5 ml – 2 ml
Urine	0.5 ml – 3 ml
Other biofluids	To be defined by the user.

2.1 Preclean the samples by centrifugation steps recommended in the table below:

Sample	Recommended protocol
Plasma	2 subsequent centrifugation steps at 4°C. 1- 10 min at 300g (save the super) 2 -20 min at 1200g (save the super)
Serum	
Urine	Centrifuge 15 min at 300g at RT
Other biofluids	To be defined by the user.

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

It is recommended to concentrate urine sample at least 5-10X to obtain a higher number of EVs. Use filter units with 100 kDa cut-off 100.

3 EV isolation:

3.1 Vortex evGAG bottle before use.

3.2 Mix the sample with evGAG using the ratio indicated in the table below:

Sample	Sample – evGAG ratio	Example	
		Sample volume	evGAG volume
Plasma	1:2 v/v	0.5 ml	1 ml
Serum	1:2 v/v	1 ml	2 ml
Urine	2:1 v/v	3 ml	1.5 ml

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

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

3.5 Centrifuge the sample at 3000g for 15 minutes at 4°C.

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

3.7 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 urine sample, step 3.6.

Protocol for Nanoparticle Tracking Analysis (NTA) of particles isolated by evGAG

1a. Prepare the sample according to the points 1 and 2 above.

2a. Follow the EVs isolation protocol (step **3.1**-**3.6**).

3a. Resuspend the pellet containing EVs in 0.25 ml Phosphate buffered saline (PBS) modified without calcium chloride and magnesium chloride.

4a**.** Proceed to NTA measures.

If working with urine, pre-treatment of uromodulin depletion is recommended before NTA: incubate the evGAG-EVs pellet with DTT (final concentration 200mg/ml) 10 minutes at 37ºC and

1000rpm. Centrifuge at 3000g, 4ºC for 15 minutes. Discard the supernatant and resuspend the pellet in the appropriate buffer.

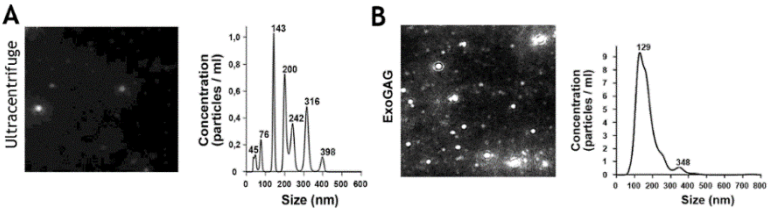

Figure 2. Nanoparticle tracking analysis (NTA) image of urine isolated EVs showing particle size (nm) and concentration (particles/ml) comparing EVs isolated by (**A**) ultracentrifugation or by (**B**) evGAG.

Protocol for Nanoparticle Tracking Analysis (NTA) of particles isolated by evGAG

1b. Prepare the sample according to the points 1 and 2 above.

2b. Follow the EVs isolation protocol (step **3.1-3.6**).

3b. Resuspend the pellet containing EVs in 0.2 ml Phosphate buffered saline (PBS) modified without calcium chloride and magnesium chloride.

4b. Incubate the resuspended EVs with antibodies (dilution according to the antibody user guide).

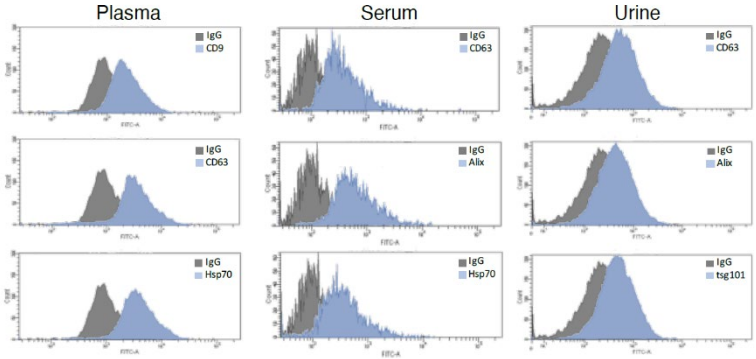

Figure 3. Cytometry analysis of EVs isolated using evGAG from 50 µl of plasma or serum and 3 ml of urine. Primary antibodies used in the example: Anti-CD9 (HBM-CD9-100), anti-CD63 (HBM-CD63-100), anti-ALIX (HBM-ALIX-100), anti-TSG101 (HBM-TSG101-100), anti-HSP70 (HBM-HSP70-100), produced by HansaBioMed Life Sciences and used according to manufacturer's guide.

Recommendations and warning

The purification kit is for research use only and for single use only and for professional use only.
The reagent contains acetic acid. Keep away from heat, hot surfaces, sparks, open flames and all other sources of ignition. Do not smoke. In the case of contact with skin or eyes, rinse with plenty of water.
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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