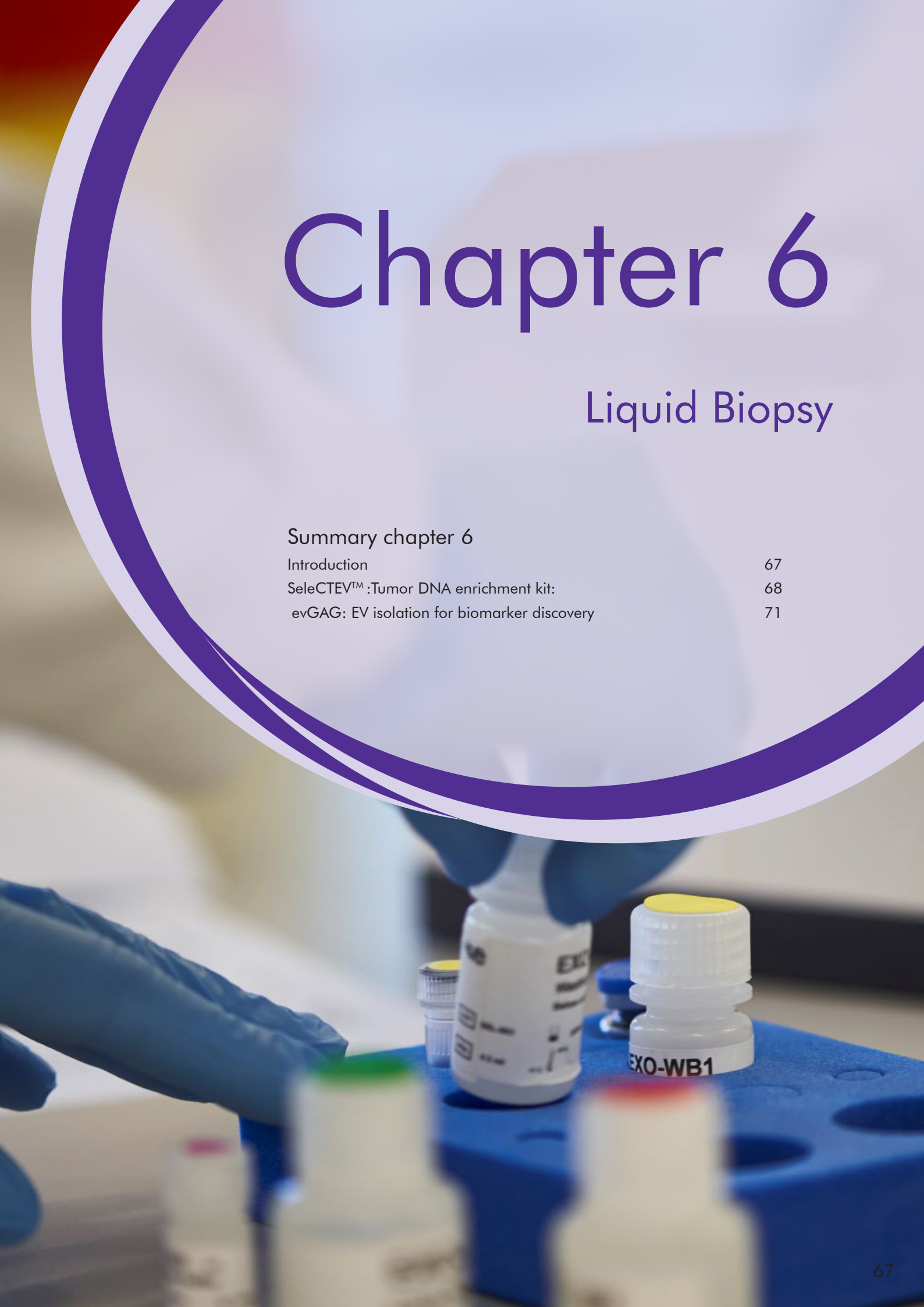
Chapter 6

Liquid Biopsy

Summary chapter 6

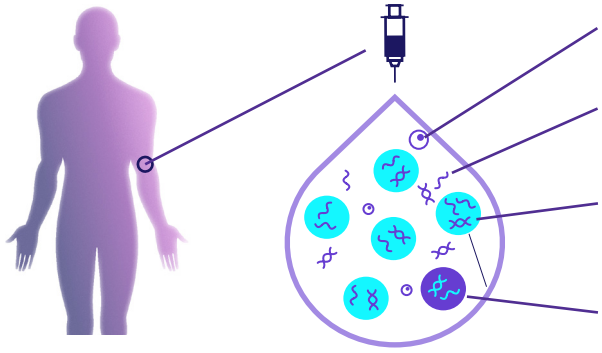


Liquid biopsy

Liquid Biopsy: introduction

Tissue biopsies are invasive and non-feasible for longitudinal monitoring of patients; moreover they do not capture the heterogeneity of the tissue or a disease of interest. Liquid biopsies are non-invasive and enable real time snapshot of the tissue homeostasis or alterations by detection of relevant biomarkers in the bloodstream. Tissue or disease specific exosomes and extracellular vesicles (EVs) can be isolated and analyzed from routine blood or urine samples featuring the ideal platform for biomarker discovery and clinical diagnostic development.

THE CHALLENGE: The major challenge and opportunity in using EVs based liquid biopsy lies in the tremendous complexity of biofluid samples, heterogeneity of exosomes and extracellular vesicles and the low abundance of specific tissue or disease markers.



CIRCULATING TUMOR CELLS (CTCSs).
Cancers may shed cells into the bloodstream. Very rare.

CIRCULATING FREE NUCLEIC ACIDS (cfRNA, cfDNA).
Mostly coming from dead cells. cfRNA is usually degraded.

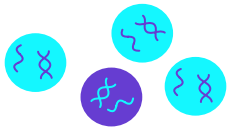
EXOSOMES AND EVs
Shuttling in the body fluids markers of parental cells.

TUMOR-DERIVED EXOSOMES AND EVs
Secreated by tumor cells in the bloodstream and body fluids.

THE SOLUTION: HansaBioMed Life Sciences provides a range of pre-analytical solutions, empowered by Exosomics s.p.a, to isolate either overall EVs or selectively enrich for specific subpopulations (i.e. tumor derived-EVs) from biofluids with high yield and purity, and further extract their DNA and RNA content. Our technologies are efficient in both biomarker discovery and confirmation studies, and comply with clinical grade analytical platforms. In clinical settings, our solutions enable ultrasensitive detection of tumor associated mutations and RNA molecules, enabling next generation of tumor screening, monitoring, staging and monitoring tests.

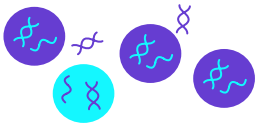
Pre-analytical solutions

Isolation of total EVs for biofluids for biomarker discovery



EV-GAG: fast isolation of EVs form biofluids

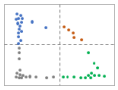
Enrichment of Tumour derived EVs and nucleic acid isolation



SeleCTEV™: Tumor DNA enrchment kit

Analytical services

Mutation analysis and biomarker screening



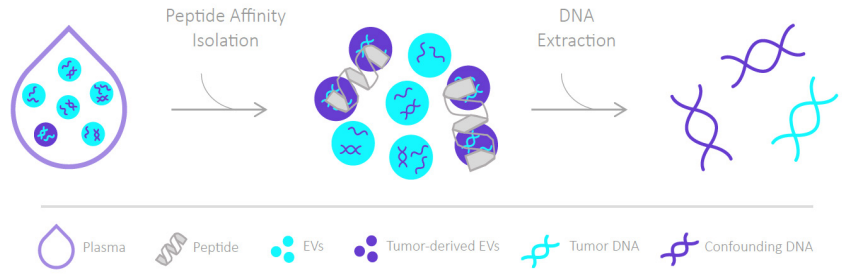
Digital PCR

Real time qPCR

NGS

SeleCTEV™ :Tumor DNA enrichment kit

SeleCTEV™ DNA Enrichment Kit allows the selective purification of circulating free DNA (cfDNA) and tumor-derived extracellular vesicles (EVs) DNA from plasma. The isolation is based on Exosomics' proprietary peptide affinity method.



Cat. Code	Description	Size
SeleCTEV: Tumor DNA enrichment kit		
HBM-EXS-DNA	Tumor DNA enrichment kit	24 reactions

Applications

- Pre-analytical purification of tumor-derived EVs. and circulating DNA (cfDNA).
- Isolated DNA suitable for the detection of actionable mutations by digital PCR (dPCR).

Characteristics

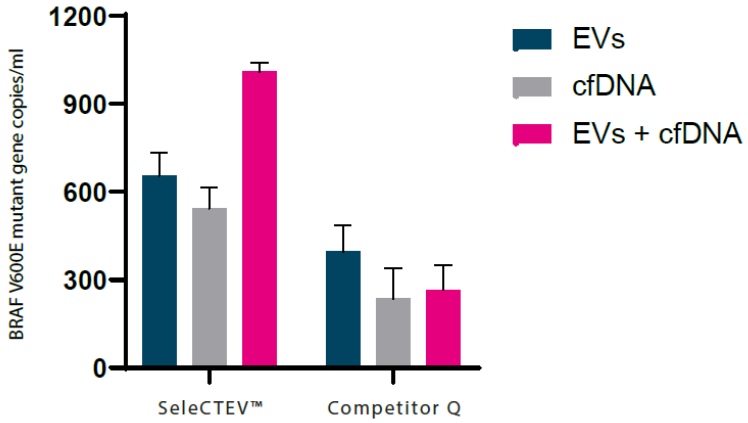
- Peptide affinity EV and DNA pull down.
- Sample type: human plasma, serum, urine.
- Sample volume: 0.5 - 2 ml of fluid.

Advantages

- Efficient enrichment of tumor-derived EVs.
- Combines EV capture and genomic DNA isolation.
- Suitable for downstream dPCR, qPCR, NGS.

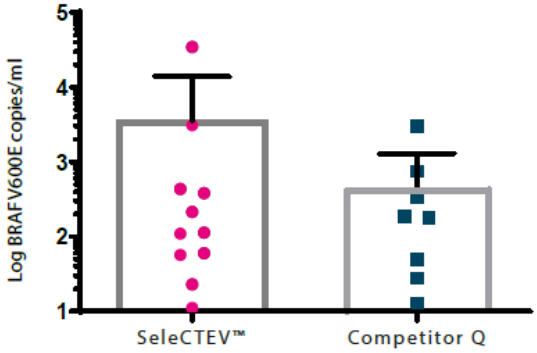
SeleCTEV™: Tumor DNA enrichment kit. Mutation recovery

Mutation-bearing EVs and cfDNA were spiked alone and together into healthy donor plasma and processed with SeleCTEV™ and Competitor Q to obtain DNA. SeleCTEV™ isolated more mutation than Competitor Q from both biological sources, suggesting that SeleCTEV™ is a more efficient way to isolate EVs and cfDNA than Competitor Q.

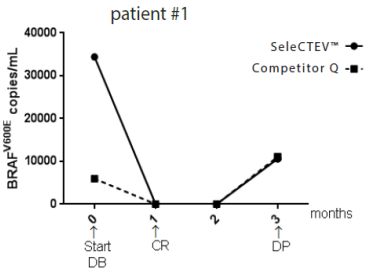


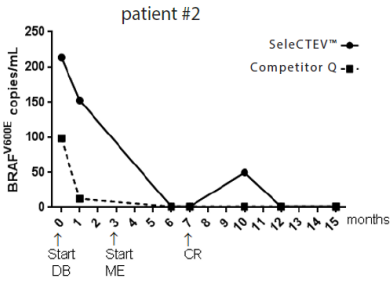
SeleCTEV™: Tumor DNA enrichment kit. Case study, metastatic melanoma patients

Plasma samples were collected from twenty patiens with BRAF V600E positive tumors and thirty patients with wild type (WT) metastatic melanoma (MM) based on tissue biopsy examination. Copies of BRAV V600E and BRAF WT were detected by digtal PCR. BRAF V600E gene copies were detected in 11 - and 8 Competitor Q - processed plasma samples of the mutant cohort.



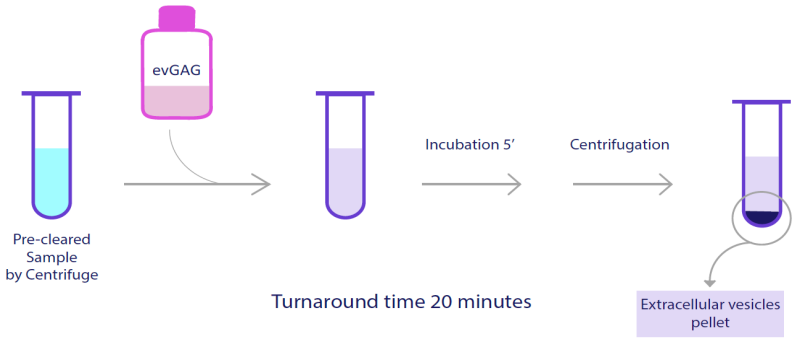
SeleCTEV™ and Competitor Q were used for monitoring BRAFV600E levels in the plasma of BRAF inhibitor-treated MM patients.
In patient #1 disease progression (DP) occurred within 3 months and was associated to rebounding levels of circulating BRAFV600E and unfavorable prognosis. In patient #2 no clinical evidence of disease progression was observed at later time points, and mutant gene copies remained low or undetectable in plasma.





evGAG: EV isolation for biomarker discovery

evGAG is a patented isolation method that allows precipitation of extracellular vesicles (EVs) from biofluids. The evGAG reaction is based on the interaction between the precipation solution and glycosaminoglycans (GAGs) in the EVs.The product is ideal for the discovery of EV associated biomarkers.



Cat. Code	Description	Size
SoRTEV: Tumor RNA enrichment kit		
HBM-EXS-GAG	Tumor RNA enrichment kit	24 reactions

Applications

- Isolation of EVs from biofluids.
- Discovery of EV associated biomarkers.
- Efficient for EVs isolation from urine

Characteristics

- Affinity isolation method.
- Sample type: Urine and diluted biofluids.
- Sample volume: 0.5 - 2 ml

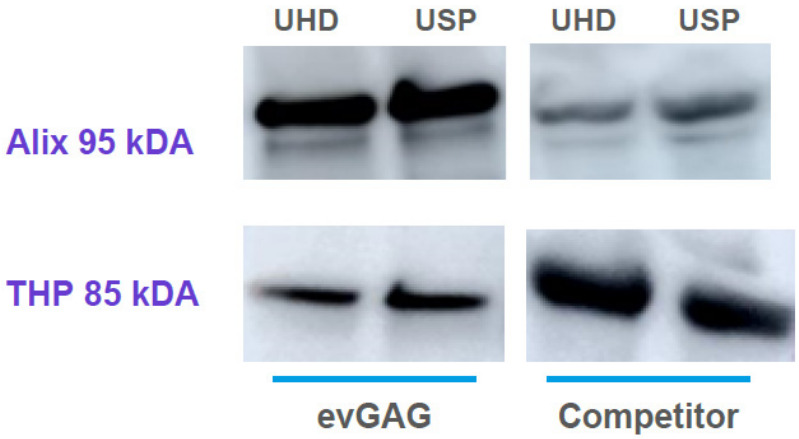
Advantages

- Rapid turnaround time (20 min).
- Small sample volume required.
- Simple procedure and high yield recovery.

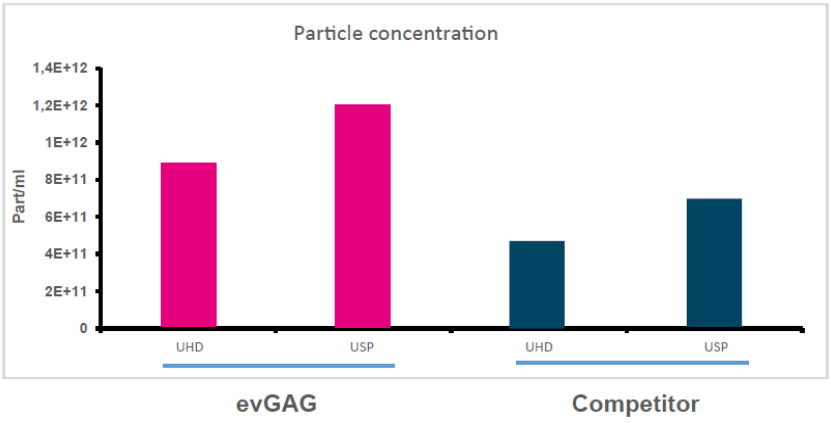
Performance of evGAG technology in isolating EVs from urine samples

Urine samples were processed with evGAG. Briefly, 1 mL of urine from healthy donor (UHD) and 1 mL of urine from healthy donor spiked-in (USP) with extracellular vesicles purified from colon cancer cell line containing KRASG13D mutation (cat nr. EXO-REF-KRAS-G13D-2) were incubated with 2 mL of evGAG each, for 5 minutes and then centrifuged at 3,000g for 15 minutes. This results in a precipitated pellet containing EVs.

Western Blot Analysis of extracellular vesicle markers Alix, confirmed that the concentration of EVs isolated by evGAG was higher compared to the competitor. On the contrary, uromoduline (THP) as not specific target is less abundant in EVs isolated by evGAG than competitor.



The pellet containing EVs was re-suspended in PBS and analyzed by Nanoparticle Tracking Analysis (NTA). Nanoparticles concentration is 2 times higher in EVs isolated with evGAG in both UHD and USP samples (8.8 x 10^{11} and 1.2 x 10^{12} respectively) than to EVs isolated with competitor in both UHD and USP samples (4.6 x 10^{11} and 6.9 x 10^{11} respectively).



EV RNA was isolated from urine using the extraction phase of SoRTEV™ and analyzed with Bioanalyzer. The results showed that EVs purified with evGAG contain the highest yield of small RNA content (<200 nt) with a main peak at 100 nt indicating an efficient extraction of small exoRNA from urine, opposed to EVs isolated with competitor kit.