# Chapter 4

## Extracellular Vesicle RNA isolation

Summary chapter 4

# Extracellular Vesicle RNA isolation

## Applications

- Direct capture and exosome RNA extraction from human biofluids and cell culture media without initial exosome purification step.
- Simultaneous miRNA and mRNA profiling (qRT-PCR, RT-PCR, microarray).

## Introduction


EVs shuttle functional RNA molecules in the target cell and EV-derived miRNAs, in particular pathogenic miRNAs, might be exploited as novel therapeutic targets or disease biomarkers , including cancer. miRNAs seem to play critical roles as transcriptional and post-transcriptional regulators of epigenetic mechanisms and cell processes and have been linked to the etiology, progression and prognosis of cancer. Similar miRNA expression patterns between tumor tissue samples and circulating exosomes have been observed.

## Advantages

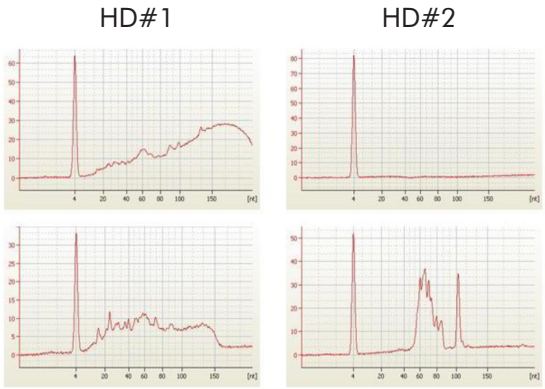
- High yield of total RNA (including small RNAs).
- Fast and user-friendly protocol.
- Small starting amount of sample (less than 1 ml).

## EV-totalRNA: RNA-EV associated isolation kit

Kit allows RNA extraction from exosomes pre-isolated with different methods (ultracentrifugation, chemical precipitation, immunocapture, size-chromatography etc.)

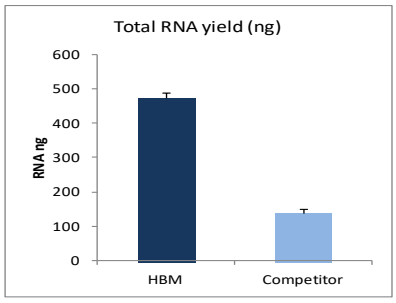
| Cat. Code | Description | Size |
|---|---|---|
| EV-totalRNA: RNA-EV associated isolation kit | | |
| HBM-RNA-B25 | RNA extraction from pre-isolated EVs | 25 reactions |
| Compatible with EVs isolated via ultracentrifuge, chemical precipitation, size chromatography, immunocapture etc. | | |

## High quality and yield of EV-associated RNAs from small volumes of sample

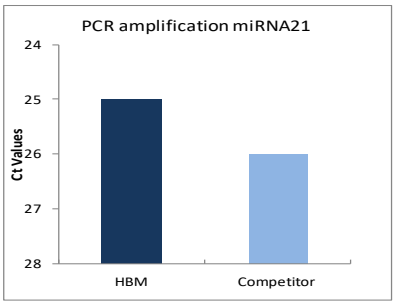



1. Electropherograms of small RNA extracted with HBM EXO-Total RNA kit and Competitor (Agilent 2100 Bioanalyzer)

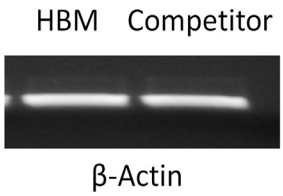
Efficiency of EV-TotalRNA kit was tested vs a competitor kit for RNA extraction from plasma-derived EVs. Extraction of total exosome RNA was performed from 100 µl of healthy donor plasma (HD #1 an #2) either with Competitor Kit and the EXO-TotalRNA Kit (HBM-LS). RNA quality was evaluated by electopherogram (Fig 1) with Small RNA microfluidic chips (Agilent 2100 Bioanalyzer). RNA yield was quantified by Nanodrop (Fig 2) and extracted RNA was subsequently retrotranscribed using the miScript II RT kit (Qiagen). miR-21 and b-actin markers were amplified by qPCR (Fig 3 and 4).




2. Nanodrop quantification of total RNA yield




3. miRNA 21 amplification by qPCR




4. β-Actin amplification by PCR