

## EV-RNA AND qPCR WORKFLOW

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### EV ISOLATION



- Consider potential RNA-binding non-EV structures (e.g proteins, lipoproteins, other EVs) present in EV sample of interest.
- Remove RNA-binding non-EV structures and purify EVs with an efficient method, such as SEC qEV columns<sup>2</sup>.
- Concentrate purified EVs if needed, with qEV Concentration kit<sup>3</sup>.
- Use relevant controls when possible.
- Store EVs appropriately to avoid EV and RNA damage (App note <sup>4</sup>).

### EV-RNA EXTRACTION



- Highly recommended: Pre-treat pure EV preparations with RNase and Proteinase K to ensure removal of non-EV RNA contaminants.
- Have a normalisation strategy for EV sample inputs (e.g by EV number, EV protein or volume)
- Optional: Add RNA spike-in to lysate for quality control of RNA extraction biases.
- Extract EV-RNA with method providing optimal purity and yields, such as qEV RNA Extraction kit<sup>5</sup>.

### EV-RNA QUANTIFICATION



- Optional: Pre-treat EV-RNA preparation with DNase to ensure removal of DNA contamination.
- Determine EV-RNA concentration with a fluorometric assay
- Analyse EV-RNA quality with an automated electrophoresis assay (enrichment of sRNAs and no large rRNA).

### RT-qPCR



- Highly recommended: Add RNA spike-in to EV-RNA for normalisation and/or RNA target quantification
- Convert EV-RNA to cDNA with RT in normalised RNA inputs. If EV-RNA concentration is unknown, normalisation can be done to EV-RNA volumes in RT or to cDNA concentration after RT.
- Amplify targets from cDNA with suitable primers, reagents and thermocycler for SYBR or Taqman approach.
- Proceed to analyse data for EV isolation controls, RNA extraction and RNA target quantification normalisers.

1 / Application note: Considerations for work with extracellular vesicle RNA, request a copy at <https://www.izon.com/application/extracellular-vesicles>

2 / qEV columns. For more information, please visit <https://support.izon.com/qev-columns>

3 / qEV Concentration kit. For more information, please visit <https://store.izon.com/products/qev-concentration-kit>

4 / Application note: How to store Extracellular Vesicles, request a copy at <https://www.izon.com/application/extracellular-vesicles>

5 / qEV RNA Extraction kit. For more information, please visit <https://support.izon.com/qev-rna-extraction-kits>