Advanta FFPE RNA Extraction Kit

Frequently Asked Questions

How much tissue should I start with?

The Advanta™ FFPE RNA Extraction Kit Protocol (PN 101-6554) is applicable for extraction of RNA from FFPE tissue sections that are 5–10 µm thick with an area of 20–200 mm^2, for a total volume of up to 2.0 mm^3 of tissue in each reaction. Due to variations in the composition of different tissues and sample types, we recommend that you test input amounts for each tissue type. Too much tissue in the extraction reaction adversely affects the RNA yield.

What types of samples could be used with the Advanta FFPE RNA Extraction Kit?

Extraction of RNA could be done using FFPE samples in forms of slides, curls and sections, core needle biopsies, or fine needle aspirates. In general, fresh-cut FFPE samples present better performance than sections cut more than a month before use.

Can I use the kit to extract DNA from FFPE samples?

No, you cannot use this kit to extract DNA.

For which applications can I use extracted RNA?

Extracted RNA can be used for any downstream application that requires RNA sample as an input for gene expression analysis and profiling, including real-time PCR and RNA-seq. Since the kit extracts high-molecular-weight RNA molecules from the tissue sample, micro-RNA analysis and other applications that require short RNA fragments (below 80 nucleotides) are not compatible with the output RNA obtained with this kit.

How long can I store extracted material?

Extracted material is stable for up to 30 days when stored at –80 °C and up to 1 week at –20 °C.

How many samples could be processed with the Advanta FFPE RNA Extraction Kit?

The kit contains sufficient reagents to perform 50 extractions.

Some master mix preparation steps decrease the number of samples that can be processed with each kit.

What is the final elution volume of the Advanta FFPE RNA Extraction Kit Protocol?

The final elution volume is 20 µL. It is recommended to retrieve no more than 18 µL to avoid drawing out beads into the final RNA suspension solution.

Can I prepare a master mix for steps that require mixing multiple reagents?

A few steps that direct the user to pipet reagents individually into each sample tube can be modified into a master mix preparation for easier pipetting when more than a few sample tubes are processed at a time. Prepare about 25% volume overage for master mixes. A master mix could be prepared for:

- Protease K digestion mixture
- Bead cleanup mixture

It is not recommended to prepare a master mix for the DNase I digestion step because adding the DNase I enzyme into the 10X DNase reaction buffer directly could impact enzyme performance.

How can I assess RNA quantity and integrity?

The quantity of RNA can be estimated by either UV absorbance at 260 nm (such as NanoDrop™) or fluorescence assay (such as Qubit®). RNA integrity can be assessed by DV_{200} as determined by any electropherogram method that analyzes the full RNA fraction, such as the Agilent® Bioanalyzer® 2100 or Fragment Analyzer.

What is DV_{200}?

DV_{200} represents the percentage of RNA fragments that are >200 nucleotides in size. This method was proposed by Illumina® scientists to more accurately assess the quality of RNA derived form FFPE tissues, which typically contain fragmented RNA transcripts due to degradation during fixation and storage.

Can I still use RNA integrity number (RIN)?

The RNA integrity number (RIN) is an algorithm for assigning integrity values to RNA measurements using the 28S-to-18S-rRNA ratio. Both the 28S and 18S rRNA are fragmented in the RNA extracted from FFPE samples. Therefore, RIN may not be a relevant metric for FFPE-derived RNA samples.

What do you recommend as FFPE RNA reference material for control?

We recommend using the 5 Fusion Multiplex RNA Negative Control (Horizon Discovery, PN HD783) for a control. The yield for a single curl may vary from lot to lot. Contact the manufacturer for detailed information on expected yield for each lot.

My extracted RNA yield is lower than expected. What could be causing that?

Lower RNA yield could be caused by:

- Loss of tissue sample during the hexadecane and/or 100% ethanol removal steps of the Advanta FFPE RNA Extraction Kit Protocol. While it is recommended to remove all the liquid in the tube, the pellet should not be disturbed. The pellet can be hard to see or might be loose. Pipette from the top of the liquid surface down to remove the supernatant and never allow the pipette tip to touch the inner surface of the tube where the pellet is. If the pellet is loose, try centrifuging again and be very careful not to agitate the tube while removing it from the centrifuge. If the pellet is still loose, it might be necessary to leave some liquid behind to avoid removing material.
- The type of magnetic stand used. Use of magnetic stands that pellet beads at the bottom rather than the side of the tube is more likely to cause loss of sample when the supernatant is pipetted out. Use the recommended brand and type of magnetic stand.

What kind of magnetic separator do you recommend?

We recommend using the DynaMag™-2 Magnet from Thermo Fisher Scientific (PN 12321D).

Can I purchase ethanol from any vendor?

Yes, you can purchase ethyl alcohol from any vendor as long as it is pure ethyl alcohol, 200 proof, molecular biology grade.

Can I purchase hexadecane from any vendor?

Yes, you can purchase hexadecane from any vendor. Hexadecane needs to be of ≥99% purity.

Why do I need to prepare fresh 80% ethanol?

Diluted ethanol evaporates over time, and its concentration changes. To ensure that the protocol is performed correctly and that results are consistent, we recommend preparing fresh 80% ethanol prior to each extraction. To prevent evaporation, make sure to close the tubes after 80% ethanol is added, particularly when processing large batches (>10 samples in parallel).

How do I contact Support?

Visit fluidigm.com/support or email support@fluidigm.com.

For technical support visit fluidigm.com/support.

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