RecoverAll™ Total Nucleic Acid Isolation Kit Guidelines for extracting RNA and DNA

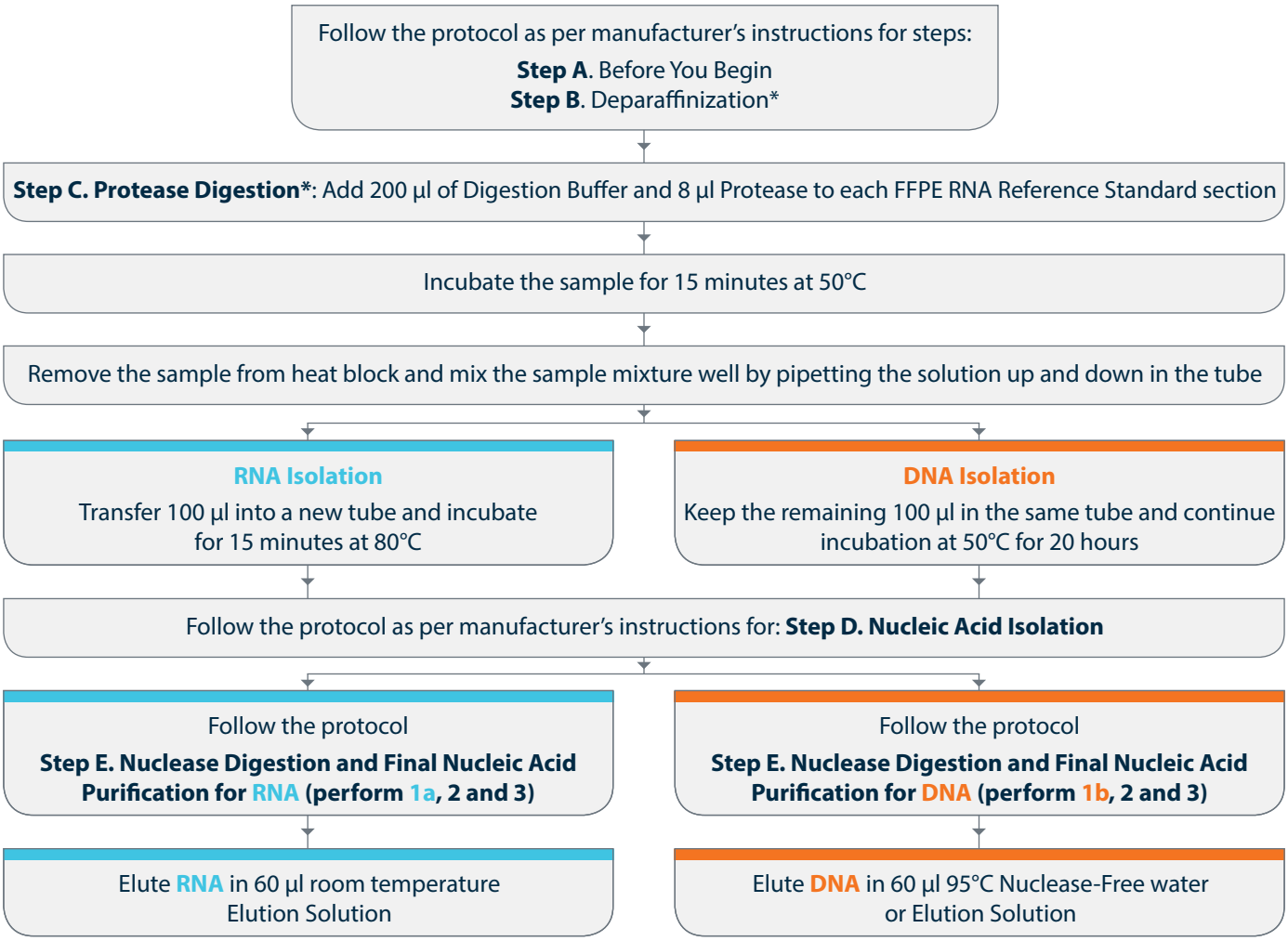
Targeted FFPE RNA Reference Standards are highly-characterized, biologically-relevant quality control material used to assess the performance of NGS, RT-PCR and RT-qPCR assays aimed at detecting gene fusions. Each section contains formalin-fixed, paraffin-embedded (FFPE) cell lines verified to contain the fusions. FFPE RNA Reference Standards allow you to evaluate your workflow integrity from pre-analytical RNA extraction through to fusion detection.

Horizon has adapted the protocol of RecoverAll™ Total Nucleic Acid Isolation Kit from Ambion® (Cat. No. AM1975) for end-users who need to extract both RNA and DNA from a single FFPE RNA Reference Standard section.

Please follow the RecoverAll™ Total Nucleic Acid Isolation Kit protocol when performing the extraction.

FFPE sections are incubated in xylene at elevated temperatures to solubilize and remove paraffin. They are then washed in ethanol to remove xylene. If both RNA and DNA are to be extracted from a single FFPE RNA Reference Standard section, the sample at the stage of Protease Digestion will be split into two aliquots for subsequent RNA or DNA extraction (see Figure 1. for the detailed step by step protocol).

Figure 1. Adapted protocol for RecoverAll™ Total Nucleic Acid Isolation Kit for RNA and DNA extraction from a single FFPE RNA Reference Standard section.



* Refer to Additional Notes on the next page

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t + 44 (0)1223 655 580 (UK) or +1 (844) 655-7800 (USA)
f + 44 (0)1223 655 581
e info@horizondiscovery.com
w www.horizondiscovery.com
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

Additional Notes

Deparaffinization

1. Perform the Deparaffinization step inside the supplied FFPE section tube – The sections are small and transferring the section from one tube to a new tube can result in it being lost and/or broken.
2. Gently flick the vial half way through the 3 minutes 50°C incubation to help dissolve the paraffin (**Step B 2d** in the protocol).
3. The section of the cell core will appear transparent after the paraffin has been dissolved in xylene. Do not tip the vial to pour away the xylene. Carefully remove it by pipetting. The cell core will turn opaque after the addition of ethanol.

Protease Digestion

1. Add 200 µl of Digestion Buffer and 8 µl of Protease directly into each sample. Gently swirl or flick the vial to mix and immerse the tissue. Briefly centrifuge to bring the section down into the solution before putting it on the heat block.
2. Gently flick the vial half way through the 15 minutes 50°C incubation and quickly place it back on the heat block.
3. At the end of the 15 minutes 50°C incubation, use a P200 pipette to pipette up and down the solution to ensure an even mix. Use the same tip to transfer 100 µl to a new tube and place the new tube on the 80°C heat block for 15 minutes (solution in this tube will be for RNA isolation). Place the original tube with the remaining 100 µl digestion mix back on the 50°C heat block and leave it for approximately 20 hours (solution in this tube will be used for DNA isolation).

Question/Observation	Recommendation
With the RecoverAll kit, there are two protocols that can be used, the RecoverAll™ Total Nucleic Acid Isolation Kit protocol (Cat. No. AM1975) and the RecoverAll™ Multi-Sample RNA/DNA Workflow protocol (Cat. No. A26069). Which is most suitable for extraction of RNA from the FFPE RNA Reference Standards?	We suggest the RecoverAll™ Total Nucleic Acid Isolation kit to extract only RNA from a single FFPE RNA Reference Standard section. The yield and the integrity of the RNA is superior when this protocol is used as compared to the Multi-Sample protocol.
Can I perform both RNA and DNA extraction from a single FFPE RNA Reference Standard section?	FFPE RNA Reference Standards have been designed and manufactured for RNA. Please use Horizon's adapted protocol of the RecoverAll™ Total Nucleic Acid Isolation kit to extract RNA and DNA from a single section. This protocol has been tested by Horizon to prove suitable for RNA and DNA isolation from a single FFPE RNA Reference Standard section.
Low RNA yield recovered.	We suggest performing the deparaffinization step using the vial in which the FFPE section is supplied in to prevent loss of material during section transfer (described in Additional Notes).
Failed RNA quantification when using the adapted protocol to extract RNA and DNA from a single FFPE RNA Reference Standard section.	We use the Qubit® RNA HS assay for measuring RNA yield. If you use a low volume of sample e.g. 2µl, it is possible the reading failed due to its low concentration. Sample input can be increased to 10µl mixed with 190µl of working solution.
Poor RNA quality recovered when using the adapted protocol to extract RNA and DNA from a single FFPE RNA Reference Standard section.	Do not incubate the sample in Digestion Buffer and Protease mixture longer than what is instructed in the RecoverAll™ Total Nucleic Acid Isolation protocol. Extending the incubation at 80°C may result in RNA degradation.

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