Instructions For Use
Veracyte FFPE RNA Extraction Kit

REF 550100

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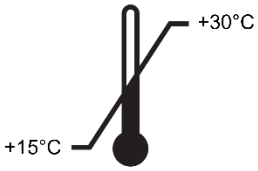
Storage condition:



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1 Table of Contents

1 INTENDED USE / PURPOSE

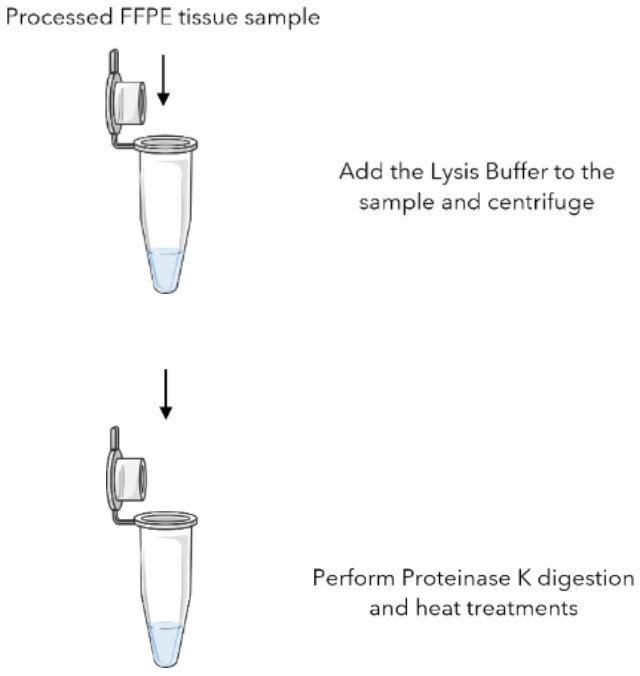
The Veracyte FFPE RNA Extraction Kit provides a set of reagents for the isolation and purification of total RNA from formalin-fixed paraffin-embedded (FFPE) human tissue samples.

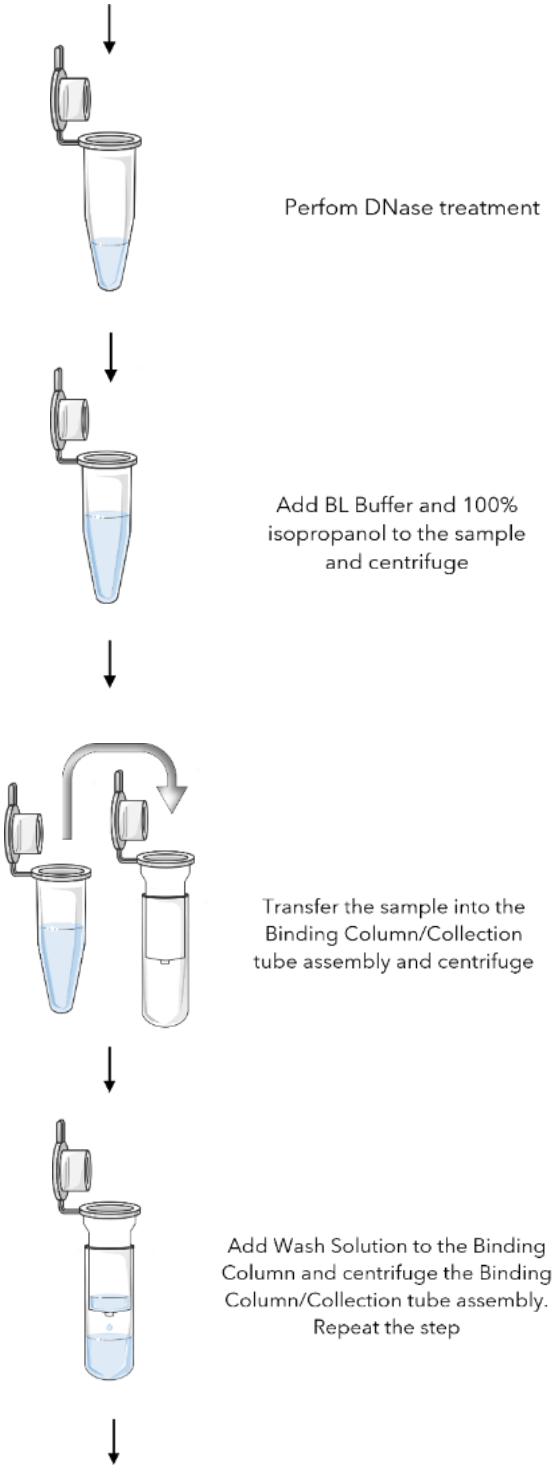
The Veracyte FFPE RNA Extraction Kit is intended for *In Vitro* Diagnostic Use and is not an automated device. It is intended to be used in a molecular biology laboratory environment by professional users such as clinical laboratory personnel specifically instructed in *In Vitro* Diagnostic procedures.

2. PRINCIPLES OF THE PROCEDURE

FFPE is a common method of archiving tissue samples that represent a vast resource for molecular profiling of clinical samples with long-term follow-up data for biomarker investigation. The Veracyte FFPE RNA Extraction Kit uses a column-based process to isolate and purify total RNA from FFPE tissue sections. It includes a DNase treatment to remove residual DNA from the purified total RNA. The recovered RNA is highly pure and suitable for many molecular biology applications commonly used in *In Vitro* Diagnostic procedures.

The RNA Isolation and Purification workflow is summarized in Figure 1.




Perfom DNase treatment
Add BL Buffer and 100% isopropanol to the sample and centrifuge
Transfer the sample into the Binding Column/Collection tube assembly and centrifuge
Add Wash Solution to the Binding Column and centrifuge the Binding Column/Collection tube assembly. Repeat the step

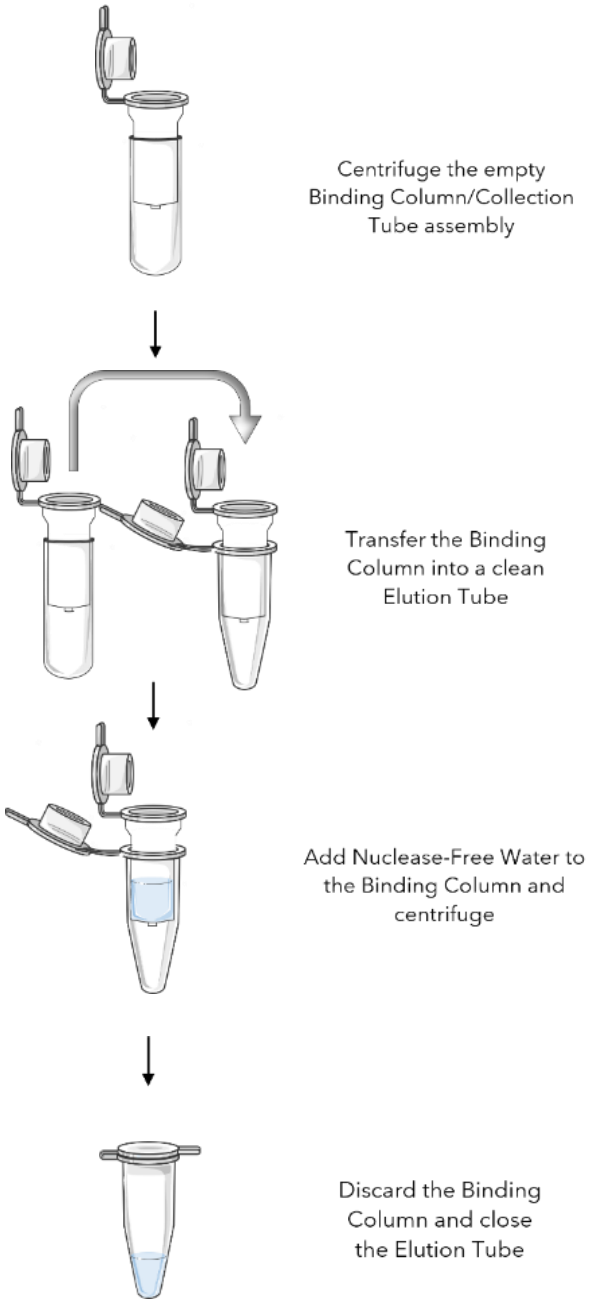


Figure 1: Principle of the Veracyte FFPE RNA Extraction Kit procedure

3. MATERIALS PROVIDED

3.1. Veracyte FFPE RNA Extraction Kit Overview

The Veracyte FFPE RNA Extraction Kit contains sufficient reagents and components to perform the isolation and purification of total RNA from 10 FFPE human tissue samples.

3.2. Veracyte FFPE RNA Extraction Kit Contents

Table 1: Veracyte FFPE RNA Extraction Kit Contents

Item	Volume/number of items
Collection Tubes	10
FFPE Binding Columns	10
Elution Tubes	2 x 5
Proteinase K (PK) Solution	250 µL
Nuclease-Free Water	1.25 mL
Lysis Buffer (LBA)	1.1 mL
DNase Buffer	80 µL
BL Buffer	3.6 mL
Wash Solution	3 mL
$MnCl_2$ 0.09 M	250 µL
DNase I (lyophilized)	1

4. KIT SHIPPING, STORAGE AND HANDLING

The Veracyte FFPE RNA Extraction Kit is shipped at room temperature (+15°C to +30°C). All components of the Veracyte FFPE RNA Extraction Kit must be stored at room temperature (+15°C to +30°C) upon receipt. Under these conditions, the Veracyte FFPE RNA Extraction Kit is stable until the stated expiration date indicated on the outer box labels.

Once opened, each reagent can be stored in their original packaging at +15°C to +30°C (except for the DNase I which once reconstituted with nuclease-free water must be stored at -15°C to -30°C), and used within three months.

For opened kits storage and to avoid multiple freeze-thaw cycles, it is recommended to dispense the DNase I once reconstituted, into single-use aliquots in nuclease-free microcentrifuges tubes (for example in nuclease-free microcentrifuges tubes with 25 µL of the reconstituted DNase I). The reconstituted DNase I must be stored at -15°C to -30°C for no more than three months. Do not freeze-thaw the reconstituted DNase I more than three times.

5. MATERIALS REQUIRED BUT NOT PROVIDED

5.1. Reagents required but not provided

1. Absolute ethanol, molecular biology grade.
2. 100 % isopropanol.

5.2. Equipment required but not provided

1. Centrifuge with a fixed angle rotor suitable with 1.5 mL and 2 mL microcentrifuge tubes.
2. Vortexer.
3. Heat blocks capable of incubation from 25°C to 80°C.

Note: Two heat blocks are required: one for the 56°C incubation and the other one for the 80°C incubation which must be carried out in a row. The metrology tolerance for the heat block/thermometer combination used during the RNA Isolation and Purification step (section 8.1) is set at +/- 2°C for the incubations done à 56°C or 80°C.

4. Micropipettes of 10 µL, 20 µL, 100 µL, 200 µL, and 1000 µL with adapted Nuclease-Free Micropipette tips and aerosol barrier.
5. Nuclease-Free microcentrifuge tube, 1.5 or 2 mL
6. Equipment required for the assessment of the RNA quality and quantity (please refer to the downstream application's Instructions For Use for details about the equipment to be used and acceptance criteria).

6. WARNINGS, LIMITATIONS AND PRECAUTIONS

6.1. General

1) The Veracyte FFPE RNA Extraction Kit is for *In Vitro* Diagnostic Use.
2) The Veracyte FFPE RNA Extraction Kit is intended to be used in a molecular biology laboratory environment by professional users such as clinical laboratory personnel specifically instructed in the techniques of *In Vitro* Diagnostic procedures.
3) Use molecular laboratory good practices to prevent cross-contamination between test samples and RNase contamination, which may negatively affect the quality of the samples.
4) Do not use the Veracyte FFPE RNA Extraction Kit with FFPE tissue sample suspended in liquid.
5) Use aerosol-resistant micropipette tips, nuclease-free reagents and consumables to avoid nucleases contamination of reagents or samples during processing.
6) Safety Data Sheet information for the Veracyte FFPE RNA Extraction Kit can be found at www.veracyte.com.
7) All biological specimens, reagents and materials (hazardous or not) should be handled as if the potential exists for transmitting infectious agents and should be disposed of with proper precautions in accordance with relevant local regulations.
8) Always wear personal protective equipment (suitable lab coat, disposable gloves, safety glasses) while handling reagents and samples.
9) Avoid reagent contact with eyes, skin, and mucous membranes.
10) Attention should be paid to expiration dates printed on the Veracyte FFPE RNA Extraction Kit labels: expired Veracyte FFPE RNA Extraction Kit should not be used.
11) Failure to store reagents under the conditions stated on the label could adversely affect Veracyte FFPE RNA Extraction Kit performance.
12) Do not mix components of the Veracyte FFPE RNA Extraction Kit across Veracyte FFPE RNA Extraction Kit lots or other extraction products. Carefully check the lot number and name on the outer box and component of the Veracyte FFPE RNA Extraction Kit before use. Functionality can only be assured for the Veracyte FFPE RNA Extraction Kit lots as provided, as the critical components are qualified in this manner during manufacturing.
13) Carefully label each microcentrifuge tube/Binding Column during the process to ensure sample traceability.
14) Equipment must be qualified, calibrated and maintained according to the laboratory's internal procedure and equipment manufacturer's recommendations.
15) The RNA isolation and purification procedure may be adapted, or slight modifications may be required depending on the downstream application. Please, refer to the downstream application's Instructions For Use. **These adaptations/modifications are accepted only if the new protocol is validated for the used downstream application.**
16) It is the responsibility of the end user to ensure that the use of the Veracyte FFPE RNA Extraction Kit (with or without procedure modification) in conjunction with the used downstream application has been validated.

6.2. FFPE sample quality and stability

1) For use of the appropriate FFPE tissue sample type during slide processing, refer to the downstream application's Instructions For Use. Before starting the slide processing procedure, attention should be paid to the FFPE tissue sample quality. It should be properly fixed.
2) For use of the appropriate number of FFPE tissue sample slides during slide processing, refer to the downstream application's Instructions For Use.
3) For any information about FFPE tissue sample stability, storage and transport conditions, refer to the downstream application's Instructions For Use.

6.3. RNA Isolation and Purification and characterisation measure

1) The DNase I is sensitive to physical inactivation. During the addition of the nuclease-free water, gently mix by swirling or flicking the vial of the solution. Do not vortex the reconstituted DNase I. For opened kits storage and to avoid multiple freeze-thaw cycles, it is recommended to dispense the DNase I once reconstituted into single-use aliquots in nuclease-free microcentrifuges tubes (for example in nuclease-free microcentrifuges tubes with 25 µL of the reconstituted DNase I). The reconstituted DNase I must be stored at -15°C to -30°C for no more than three months. Do not freeze-thaw the reconstituted DNase I more than three times.
2) For RNA quality and quantity acceptance criteria and the method of measurement, refer to the downstream application's Instructions For Use.
3) For purified RNA storage and transport conditions and duration refer to the downstream application's Instructions For Use.
4) For potentially interfering substances impacting downstream application function, refer to the downstream application's Instructions For Use.
5) For cross-contamination events susceptible to impact downstream application reliability, refer to the downstream application's Instructions For Use.

7. SAFETY AND DISPOSAL

All biological specimens should be disposed of with proper precautions in accordance with relevant local regulations.

All chemical materials should be disposed of according to their Safety Data Sheets, relevant local regulations and/or your institution's guidelines for hazardous disposal.

Always wear personal protective equipment (gloves, safety glasses, lab coat) while handling reagents and samples. For more information, please consult the appropriate safety data sheets (SDSs).

Safety Data Sheet information for the Veracyte FFPE RNA Extraction Kit can be found at www.veracyte.com.

8. RNA ISOLATION AND PURIFICATION PROCEDURE

Before starting the RNA isolation and purification procedure:

- Refer to the downstream application's Instructions For Use for the slide processing procedure.

Warning: The slide processing procedure must have been validated for its use with the Veracyte FFPE RNA Extraction Kit. Do not use the Veracyte FFPE RNA Extraction Kit with FFPE tissue sample suspended in liquid.

- Prepare solutions as follow:

1) Add 12 mL of absolute ethanol to the Wash Solution bottle.

Note: After adding absolute ethanol, mark on the bottle that you have performed this step and the date. When capped tightly, this reagent is stable at room temperature for three months.

2) Add 275 µL of Nuclease-Free Water to the lyophilized DNase I vial. Store the reconstituted DNase I on ice until use.

Warning: The DNase I is sensitive to physical inactivation. During the addition of the nuclease-free water, gently mix by swirling or flicking the vial of the solution. **Do not vortex the reconstituted DNase I**. For storage of opened kits and to avoid multiple freeze-thaw cycles, it is recommended to dispense the DNase I once reconstituted into single-use aliquots in nuclease-free microcentrifuges tubes (for example in nuclease-free microcentrifuges tubes with 25 µL of the reconstituted DNase I). The reconstituted DNase I must be stored at -15°C to -30°C for no more than three months. **Do not freeze-thaw the reconstituted DNase I more than three times.**

8.1. RNA Isolation and Purification

The RNA isolation and purification procedure may be adapted, or slight modifications may be required depending on the downstream application. Please, refer to the downstream application's Instructions For Use. **These adaptations/modifications are accepted only if the new protocol is validated for the used downstream application.**

Sample Lysis

1. Add 100 µL of Lysis Buffer to the microcentrifuge tube containing the processed FFPE tissue sample.

Warning: Carefully label each microcentrifuge tube during the process to ensure sample traceability.

2. Centrifuge the microcentrifuge tube containing the sample at 10,000 x g for 15 seconds at room temperature.
3. Add 10 µL of Proteinase K to the microcentrifuge tube containing the sample. Mix by gentle pipetting.
4. Incubate the microcentrifuge tube containing the sample at 56°C for 15 minutes.
5. Incubate the microcentrifuge tube containing the sample at 80°C for 1 hour.
6. Remove the microcentrifuge tube containing the sample from the 80°C heat block and place it on ice for 1 minute to cool. Then, place the microcentrifuge tube containing the sample at room temperature for at least 2 minutes until the DNase treatment is performed.
7. Centrifuge briefly and proceed immediately to the DNase treatment.

DNase treatment

8. While the microcentrifuge tube containing the sample is equilibrating at room temperature, prepare or thaw enough of the reconstituted DNase I aliquots. Store the reconstituted DNase I on ice until use.
9. Prepare the DNase treatment mix: for each isolation to be performed, combine the following ingredients in a nuclease-free microcentrifuge tube:
    i. 14.3 µL of $MnCl_2$ 0.09M. Mix by gentle pipetting.
    ii. 7.7 µL of DNase Buffer. Mix by gentle pipetting.

iii. 11 µL of the reconstituted DNase I. Mix by gentle pipetting.

WARNING: Prepare the DNase treatment mix immediately before use. It must be prepared fresh for each RNA isolation process

Prepare only the amount of DNase treatment mix required for the number of processed samples.

These volumes already include the extra 10% volume, which corresponds to the dead volume.

10. Add 30 µL of freshly prepared DNase treatment mix directly to the microcentrifuge tube containing the sample. Mix by gentle pipetting.
11. Incubate the microcentrifuge tube containing the sample for 15 minutes at room temperature (25°C). It is recommended to use a heat block setting at 25°C for a better temperature control.
12. Proceed immediately to the RNA Binding step.

RNA Binding

13. Add 325 µL of BL Buffer to the microcentrifuge tube containing the sample.
14. Add 200 µL of 100% isopropanol to the microcentrifuge tube containing the sample. Vortex briefly to mix.
15. Centrifuge the microcentrifuge tube containing the sample at 10,000 x g for 15 seconds at room temperature.
16. For each microcentrifuge tube containing the sample to be processed, place a Binding Column into one of the Collection Tubes and transfer the supernatant to the Binding Column/Collection Tube assembly.

    Warning:
    Do not disturb the pellet during the supernatant's transfer.
    Carefully label each Binding Column during the process to ensure sample traceability. Cap the column. Discard the microcentrifuge tube with the pellet.

17. Centrifuge the Binding Column/Collection Tube assembly at 10,000 x g for 30 seconds at room temperature.
18. Discard the flowthrough and reinsert the Binding Column into the same Collection Tube.
19. Proceed immediately to the RNA Washing step.

RNA Washing

20. Add 500 µL of 1X Wash Solution to the Binding Column. Cap the column.
    Warning: Make sure that absolute ethanol was added to the Wash Solution before use.
21. Centrifuge the Binding Column/Collection Tube assembly at 10,000 x g for 30 seconds at room temperature.
22. Discard the flowthrough and reinsert the Binding Column into the same Collection Tube.
23. Add 500 µL of 1X Wash Solution to the Binding Column. Cap the column.
    Warning: Make sure that absolute ethanol was added to the Wash Solution before use.
24. Centrifuge the Binding Column/Collection Tube assembly at 10,000 x g for 30 seconds at room temperature.
25. Discard the flowthrough and reinsert the Binding Column into the same Collection Tube. Cap the column.
26. Centrifuge the Binding Column/Collection Tube assembly at 16,000 x g for 3 minutes to dry the column.
27. Discard the flowthrough.

28. Proceed immediately to the RNA Elution step.

RNA Elution

29. Discard the Collection Tube and transfer the Binding Column into a clean Elution Tube.
30. Add 30 µL of Nuclease-Free Water to the Binding Column and cap the column.
31. Centrifuge at 16,000 x g for 1 minute at room temperature. Remove and discard the Binding Column.
32. Cap the Elution Tube.

Note: For RNA quantification and purity method and related acceptance criteria as well as for purified RNA storage condition and duration, consult the downstream application's Instructions For Use.

9. QUALITY CONTROL

Each lot of the Veracyte FFPE RNA Extraction Kit has been tested against predetermined specifications to ensure consistent product performance. Functionality can only be assured for the Veracyte FFPE RNA Extraction Kit lots as provided, as the critical components are qualified in this manner during manufacturing.

10. TROUBLESHOOTING GUIDE

This troubleshooting aims to help solve any problems that may arise using the Veracyte FFPE RNA Extraction Kit. For questions not addressed here, please contact technical support at dxsupport@veracyte.com.

The purified RNA has a low yield and/or is of poor quality:

Cause	Measures to be taken
Absolute ethanol was not added to the Wash Solution prior to use.	Check that absolute ethanol was added to the Wash Solution (record shall have been done on the Wash Solution bottle label).
The Veracyte FFPE RNA Extraction Kit was stored under deleterious condition.	Check that each reagent was stored in its original packaging at +15°C to +30°C (except for the DNase I which once reconstituted with nuclease-free water must be stored at -15°C to -30°C).
The Veracyte FFPE RNA Extraction Kit reagents are expired.	Check the expiration dates printed on the Veracyte FFPE RNA Extraction Kit label. Check that the kit was opened and that the DNase I was reconstituted and the wash buffer prepared less than three months ago. Check that the reconstituted DNase I has not been freeze-thaw more than three times
RNases have been introduced during sample processing or quantitation.	To avoid introducing contaminating RNases, gloves should be always worn during the extraction procedure. Use aerosol-resistant micropipette tips, nuclease-free reagents and consumables.

The purified RNA gives no result or incorrect result with the downstream application

Cause	Measures to be taken
The FFPE samples were improperly prepared or are too old or were stored under deleterious condition.	Check in downstream application Instructions For Use the FFPE recommendation regarding FFPE sample preparation, FFPE sample stability and FFPE sample storage conditions.
A wrong FFPE sample type was used or not enough/too much FFPE slides were used.	Check in downstream application Instructions For Use the FFPE sample type claimed and the number of FFPE slides recommended.
The FFPE samples contain an interfering substance.	Check in downstream application Instructions For Use the FFPE information regarding interfering substance. Partially dissolved paraffin.
The presence of genomic DNA interferes with the downstream application functioning.	Check that the DNase I has been properly reconstituted and once reconstituted, has been stored at -15°C to -30°C for no more than three months and has not been freeze-thaw more than three times. The DNase treatment mix must be prepared fresh for each RNA isolation process. The DNase treatment mix mustn't be stored nor vortexed.
The Veracyte FFPE RNA Extraction Kit is not suitable to be used with the downstream application.	Ensure that the Veracyte FFPE RNA Extraction Kit has been validated with the downstream application (refer to the downstream application Instructions For Use).

11. SYMBOLS AND DEFINITIONS

The following symbols appear in the Instructions For Use or on the packaging and labeling:

Symbol	Symbol definition
	Contains sufficient for <n> tests
	CE mark
	Use by/Expiration date
	*In Vitro* Diagnostic Medical Device
	Lot number/batch code
	Catalogue number
	Global Trade Item Number
	Temperature limit
	Manufacturer
	Consult Instructions For Use
	Caution

12. CONTACT INFORMATIONS

Please be aware that if a serious incident occurs in relation to this medical device, whatever the affected person (user and/or patient), the aforesaid serious incident should be reported to the medical device manufacturer and/or its authorized representative and the competent authority of the Member State in which the affected person is established. Please consult your local regulations for serious incidents reporting.

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Website: www.veracyte.com

13. DOCUMENT REVISION HISTORY

Revision	Description
Version 03	**The following modifications are implemented:** **Section 1: The word "manual" is removed from the intended use.** **Section 4: In use stability clarified (limited to three months).** **Section 5.2: Metrology tolerance for the heat block/thermometer indicated – reminder added: equipment to use for RNA quality and quantity determination depends on the downstream IVD procedure.** **Section 6: Warning added as the kit must not be used with FFPE tissue sample suspended in liquid – any modification of the protocol must be validated considering the downstream application (same reminder added in 8.1).** **Section 6.3 & 8: stability of the kit once opened is reminded.** **Section 8.1: Warnings added as each microcentrifuge tube and binding column must be labelled to ensure sample traceability - Instructions update for the DNAse treatment mix preparation (warning in step 9) – note in step 9 was changed to Warning.** **Section 10: Update of measures to implement for the expired reagents and genomic DNA interference cases.**
Version 02	**Second version for commercialization**
Version 01	**Creation of the document – first version for presentation before commercialization.**