Asuragen®

RNA Retain®

Instructions for Use
For In Vitro Diagnostic Use

REF 84001 IVD

DI 00863840000285

Σ 24

Asuragen, Inc.
2150 Woodward St.
Suite 100
Austin, TX 78744
USA

Table of Contents

Intended Use

The RNA*Retain*® device is a single-use, prefilled container intended for the collection, storage, and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing.

For Professional Use Only.

Background Information

The utility of the RNA*Retain* solution is to maintain high quality cellular RNA for downstream molecular testing. The collection of fresh tissue specimens into the RNA*Retain* device eliminates the need to immediately process these specimens, enabling RNA extraction and molecular testing at a later time and/or transport to a different location. RNA*Retain* eliminates the need to flash-freeze specimens and to keep specimens frozen throughout storage and transport, a process that is costly and involves manipulation of potentially hazardous agents. It has been demonstrated that the RNA purified from fresh tissue specimens stored in RNA*Retain* or flash-frozen can be used in the same downstream applications[1]. RNA*Retain* also eliminates the need for formalin fixation, the most common method of clinical tissue preservation, that is both hazardous to work with and known to cross-link and sometimes degrade the nucleic acids rendering them unacceptable for specific downstream molecular applications.

Test Principle

The RNA*Retain* device consists of an aqueous, hypertonic tissue preservation solution that is provided in a single-use, non-sterile vial intended to serve as the container for the collection, storage and transport of fresh tissue specimens. The ability of the solution formulation to preserve fresh tissue and nucleic acids within the tissue is due to its rapid permeation of tissue to protect cellular nucleic acids from nucleases that would otherwise lead to rapid degradation. The demonstrated inhibitory effect of the RNA*Retain* solution on the growth of bacteria, yeast and mold protects the specimen from inadvertent microbial contamination during storage or usage of the device.

Kit Components

RNA*Retain* Ordering Options

Item #	Volume	Storage Temp
84001	24 × 1.0 mL	18 to 25 °C

Handling and Storage

- Store the reagents in a cool dry place before use.

Number of Reactions

- Each RNA*Retain* device is for single use. Boxes are available containing 24 vials.

Reagent Stability

- The product will maintain performance through the expiration date printed on the label when stored under the specified conditions.

Warnings and Precautions

- Use proper personal protective equipment. Wear appropriate protective eyeglasses, protective gloves, and protective clothing when working with RNA*Retain*®.
- Follow Universal Precautions when handling human samples[2, 3].
- Treat used RNA*Retain* vials as potentially infectious. Upon removing specimens from RNA*Retain*®, dispose of all materials that have come in contact with specimens and reagents in accordance with federal, state and local regulations.
- RNA*Retain* solution should not come in contact with bleach or products containing bleach.
- Closely follow procedures and guidelines provided to ensure adequate RNA stabilization. Any deviation from the outlined procedures and guidelines may affect optimal performance.
- Do not use the RNA*Retain* after its labeled expiration date.
- RNA*Retain* vials are for single use only. Do not reuse.
- RNA*Retain* RNA stabilization properties are optimal at a specimen to solution ratio of 1:10 or less. For example, do not add a specimen that is more than 0.5 cc in volume to a vial containing 5 mL of solution. If the specimen to solution ratio is greater than 1:10, inefficient penetration of the stabilization solution and degradation of RNA during storage or transport may occur.
- Insoluble materials that might be used with collection methods (such as contrast dyes) or histological sample processing methods are not compatible with RNA*Retain*.
- Failure to fully immerse the tissue into the preservation solution in a timely manner may lead to delayed nuclease inactivation resulting in degradation of cellular RNA prior to solution penetration.
- Do not freeze specimens prior to placement into RNA*Retain*.
- If precipitate is observed in an unused RNA*Retain* vial, do not use.

RNA*Retain* Protocol

1. Remove the number of RNA*Retain* vials from the reagent storage box equivalent to the number of specimens to be collected.
2. Immediately after specimen acquisition completely immerse specimen into an RNA*Retain* collection vial containing preservation solution to allow thorough penetration of reagent. The ratio of the specimen volume to the solution volume stated on the collection vial label should be 1:10 or less.
3. Tissue specimens should be incubated in RNA*Retain* for at least 12 hours prior to freezing.
4. Specimens can then be transported or stored in RNA*Retain* for:
    - up to 3 years at -15 to -30 °C
    - up to 30 days at 2 to 8 °C
    - up to 7 days at 18 to 25 °C, or
    - up to 3 days at 35 to 39 °C.
5. Remove specimens from RNA*Retain* prior to nucleic acid extraction.

Performance Characteristics

Stability of specimens stored in RNA*Retain*

MCF-7 human breast adenocarcinoma cell aliquots were collected in RNA*Retain*® and stored over multiple temperatures and time-points. Total RNA was subsequently isolated from each of the triplicate aliquots. Total RNA purity was measured and RNA integrity was assessed. The average RNA purity and integrity values were determined from triplicate measurements and are shown in Table 1. These results demonstrate the ability of RNA*Retain* to preserve intracellular RNA within cell specimens stored as described below. All RNA samples met the acceptance criteria for RNA integrity (28S:18S) ≥ 1.0 and RNA purity (A260/A280) ≥ 1.6.

Table 1. Quality of total RNA recovered from MCF-7 cells stored in RNA*Retain* over multiple temperatures and time-points.

Temp (°C)	Time	Integrity (28S:18S)	Purity (A_{260}/A_{280})
35 to 39	3 Days	1.3	1.94
18 to 25	7 Days	1.6	2.02
2 to 8	30 Days	1.2	1.98
-15 to -30	3 Years	1.7	1.99

Precision (reproducibility/repeatability)

Ten adjacent pairs of breast tissues were collected from each of 3 subjects' surgically removed sections. From each pair, one was stored in RNA*Retain* at 2-8°C for 12 to 18 hours; the other was frozen in liquid nitrogen and stored at ≤-70°C. The RNA*Retain* and flash-frozen stored specimens were then shipped overnight on blue ice or dry ice, respectively. The samples were processed immediately upon receipt for RNA purification and RNA analysis. The average integrity, purity and yield values for each subject (10 replicates), together with the associated standard deviations (SD) are presented in Table 2. There was no significant difference between tissues from various storage methods, indicating that RNA quality from human breast tissue stored in RNA*Retain* is equivalent to flash-frozen specimens. All RNA samples met the acceptance criteria for RNA integrity (28S:18S) ≥ 1.0 and RNA purity (A260/A280) ≥ 1.6.

Table 2. Mean results for the 3 breast tissue replicates.

		Integrity (28S:18S)		Purity (A_{260}/A_{280})		Yield
Subject	Storage	AVG	SD	AVG	SD	AVG
1	RNA*Retain*	1.5	0.3	1.97	0.03	148
	Frozen	1.6	0.2	1.92	0.06	131
2	RNA*Retain*	1.6	0.2	2.03	0.04	62
	Frozen	1.5	0.2	2.01	0.11	43
3	RNA*Retain*	1.5	0.2	1.96	0.14	101
	Frozen	1.5	0.1	1.96	0.09	81

Analytical performance of Agendia MammaPrint® with RNA*Retain*

Precision/Reproducibility – 5 tumor samples were isolated in duplicate. Over multiple days the ten isolations were processed through the test result for MammaPrint®. Results demonstrated no statistically significant difference between isolations. Results were equivalent to original test results and interpretation using fresh frozen samples[4].

Stability of MammaPrint outcomes in RNA*Retain*® - Shipment of a tumor in both RNA*Retain* and fresh frozen was performed to determine stability and variance. Both samples were labeled 5 times and processed through the test result for MammaPrint. Incorporation of Cy5 and Cy3 were higher with the RNA*Retain* specimens and variance in indices were smaller than for fresh frozen samples with no significant differences between MammaPrint index acceptable limit of variation[4].

Method Comparison study of Agendia MammaPrint® with RNA*Retain*

Results from tissue preserved with RNA*Retain* was compared to results obtained from fresh frozen tissue using the MammaPrint device. The study samples were collected and shipped in RNA*Retain* from 20 hospitals. Thirty-three breast tumor samples of which one part of the sample was immediately snap-frozen in liquid nitrogen and stored at -70°C, the remaining portion was stored in RNA*Retain* for 3 to 5 days at room temperature. Afterwards the tissue was removed from the preservation solution, snap frozen and stored at -70°C. Another set comprised of 18 tumors of which two parts were available for research that were immediately snap frozen and stored at -70°C. RNA isolation and DNAse treatment were performed in this same period. All samples were hybridized on MammaPrint microarrays, and passed all sample, labeling and hybridization QCs. Results from the paired RNA*Retain* and frozen samples were analyzed and shown to have a median difference of 0.070. The Pearson correlation (0.94) and regression analysis indicate a high similarity ($R^2 = 0.90$). This finding is similar to the results of a series of tumors of which two frozen samples were available and were collected in the same time period. The median difference in MammaPrint Index was 0.105. A comparison of the differences in both series (RNA*Retain*-frozen vs. frozen-frozen) showed no significant difference (t-test, p=0.57) indicating no variation is introduced by RNA*Retain*.

Disclaimers

- This product is intended for in vitro diagnostic use.
- This product may not be resold or modified for resale without the written approval of Asuragen®.
- Asuragen® and RNA*Retain*® are registered trademarks of Asuragen, Inc.
- TO THE EXTENT PERMITTED BY APPLICABLE LAW, IN NO EVENT SHALL ASURAGEN BE LIABLE IN ANY WAY (WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT LIABILITY OR OTHERWISE) FOR ANY CLAIM ARISING IN CONNECTION WITH OR FROM THE USE OF THIS PRODUCT.

References

1. Chowdary D, Lathrop J, Skelton J, et al. Prognostic gene expression signatures can be measured in tissues collected in RNA*later* preservative. J Mol. Diagn 2006 8:31-9.
2. CLSI M29-A3. Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline – Third Edition. 2005, Vol. 25 No. 10.
3. OSHA. Occupational Safety and Health Standards, Toxic and Hazardous Substances, Bloodborne pathogens. 1991, PN 1910, SN 1910.1030, Appendix A.
4. K070675 510(k) Agendia MammaPrint Substantial equivalence determination decision summary by FDA. Comparison of RNA*Retain*® to Fresh Frozen breast tissue specimens. From FDA website: http://www.accessdata.fda.gov/cdrh_docs/reviews/K070675.pdf

Technical Support and Ordering Information

- For technical support, please call 1.512.681.5200 or email support@asuragen.com.
- For ordering additional RNA*Retain*, please call 1.512.681.5200, fax 1.512.681.5201, or email support@asuragen.com and request catalogue number 84001.

Appendix A: Glossary of Symbols

Symbol	Description
	Contains sufficient for <n> tests
	Consult instructions before use
	Use by
	*In vitro* diagnostic medical device
	Catalog number
	Batch code
	Temperature limitation
	Manufactured by
	Do not reuse