RNA*wait*™

RNA STABILIZING SOLUTION FOR TISSUE

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For research use only / For professional use only / Not for diagnostic purposes

INTENDED USE / GENERAL INFORMATION

RNA*wait*™ is a tissue collection and storage solution that quickly permeates fresh tissues to stabilize RNA. It streamlines operations by eliminating the need for immediate downstream processing or freezing of the sample. Instead, the tissue can be submerged in RNA*wait*™ and stored. Hence, the RNA extraction step can wait.

Tissues in RNA*wait*™ can be stored for extended periods while preserving RNA: one month at 4 °C, one week at 25 °C, or one day at 37 °C. When tissue is taken out of RNA*wait*™, it can be stored at -20 °C or lower for an indefinite period.

NOTE

DNA and proteins are also preserved in RNA*wait*™. Notably, proteins will be denatured and thus only suitable for applications such as western blotting, 2D electrophoresis, or mass spectrometry, but not for applications requiring native protein (rapid antigen testing, ELISA).

COMPOSITION

The product is a hypertonic mild acidic salt solution containing ammonium sulphate and EDTA. An MSDS can be downloaded from the website www.inactivblue.com.

MATERIAL INCLUDED – REFERENCE CODES

RW_0001	72x 1 mL of RNA*wait*™
RW_0100	1x 100 mL of RNA*wait*™
RW_0500	1x 500 mL of RNA*wait*™

MATERIAL NEEDED, BUT NOT PROVIDED

Sample collection devices

SPECIMEN TYPE

RNA*wait*™ is suitable for stabilizing RNA in most fresh tissues, cultured cells, and microorganisms.

It is strongly recommended to first test the sample type of interest with RNA*wait*™.

INSTRUCTIONS FOR USE

I. Cut fresh tissue into small pieces (< 100 mg, < 5 mm in at least one dimension), and initially store in 1 mL of RNA*wait*™ for 8-24 hours (e.g., overnight) at 4 °C.

NOTE

If fresh tissue in RNA*wait*™ cannot be immediately conditioned at 4 °C, store in a properly closed (screw capped) tube for a few hours on ice-water (0 °C).

II. For continued storage (after initial incubation in RNA*wait*™ at 4 °C),
   - keep in RNA*wait*™ at 4 °C up to 30 days, at 25 °C up to one week, or at 37 °C for maximum 1 day; after this period, either proceed with RNA extraction, or freeze for more extended storage
   - take tissue out of RNA*wait*™ (dry with a paper towel) and freeze at -20 °C or lower (indefinitely). Multiple freeze-thaw cycles will not impact the RNA quality.

III. For RNA extraction, remove tissue from RNA*wait*™ (if not frozen yet; dry with a paper towel) or let it thaw in the fridge (~1 hour) or at room temperature. Then, follow the instructions of the RNA extraction kit.

LIMITATIONS

Samples that are poorly impregnated (like waxed plant tissue, or bone) may not be adequately preserved.

Some tissues may need to be cut thinner (e.g. < 2 mm in at least one dimension). During our validation studies, murine kidneys were cut in half (~2 mm thickness), while complete hearts (~5 mm) were preserved in RNA*wait*™.

STORAGE AND STABILITY OF RNA*WAIT*™ IN ITS ORIGINAL PACKAGING

- Store the product between 15 °C and 25 °C.
- If precipitation is seen, heat to 37 °C and agitate to redissolve it.
- Keep away from direct (sun) light.
- Dispose of RNA*wait*™ in accordance with local regulations.

INSTRUMENT/ SYSTEM COMPATIBILITY

RNA*wait*™ validation studies were done using MagMAX miRVana Total RNA Isolation Kit (#A27828, Thermo Fisher Scientific) upon bead-based homogenization of the tissue in lysis buffer. Most commercial RNA isolation kits and standard RNA isolation protocols are compatible with RNA*wait*™.

PERFORMANCE DATA

TISSUE STABILIZATION

RNA integrity (RIN values) and molecular stability (RT-qPCR analysis of *Anapc5* and *Cd81*) have been studied in murine heart and kidney tissue. When prepared in RNA*wait*™ as described in the instructions for use, the following performance characteristics are guaranteed, dependent on the indicated storage conditions:

	4 °C	25 °C	37 °C	freeze/thaw cycles
intact RNA (RIN values)	30 days	7 days	1 day	5x
molecular stability (RT-qPCR)	30 days	7 days	1 day	5x

OTHER CHARACTERISTICS

- RNA*wait*™ does not disrupt the structure of a tissue.
- Tissue impregnated in RNA*wait*™ can be removed from the solution, cut into smaller pieces, and returned to the solution, if needed.
- Study data confirms preservation of tissue histology after removal from RNA*wait*™ and fixation in buffered formalin, embedding in paraffin, and hematoxylin/eosin staining. A more pronounced eosinophilic staining and more loose extracellular matrix may be observed.

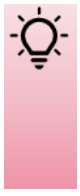
RNA QUALITY CONTROL

RNA INTEGRITY

Intact RNA is visualized by clear presence of ribosomal RNA bands. RNA integrity values (RIN) depend on measurement instrument type, RNA concentration, and RNA extraction method. Residual genomic DNA may also negatively affect the RIN value.

NOTE

Low RNA concentration and the presence of small RNAs (e.g. microRNAs) generally result in somewhat lower RIN values.



NOTE

While RIN ≥ 5 is recommended for RT-qPCR quantification (Becker et al., Methods, 2010), other studies indicate that the level of acceptable RNA integrity depends on factors such as the expression difference, the target abundance, the intra-group variability, the sensitivity to degradation of the target, and the gene expression measurement method (Vermeulen et al., Nucleic Acids Research, 2011).

RNA CONCENTRATION & PURITY

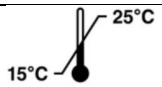
RNA concentration can be determined using UV spectrophotometry, fluorescent dye binding, or (microfluidic) electrophoresis. While frequently reported, 260/280 and 260/230 nm absorbance ratios are not reliable metrics of nucleic acid purity, especially for concentrations ≤ 20 ng/µL and when eluted in water (Wilfinger et al., Biotechniques, 1997; Shim et al. Biopreserv Biobank, 2010; Okamoto et al., Int J Mol Med, 2000; Unger et al., Anal Biochem, 2019; Koetsier and Cantor, New England Biolabs Technote, 2019: https://bit.ly/42UC6wP).

WARNINGS AND PRECAUTIONS

Avoid inhalation and ingestion. Wash hands and other exposed areas with mild soap and water before eating, drinking, or smoking and when leaving work. Provide good ventilation in process area to prevent formation of vapor.

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting viruses. Always wear protective clothing when handling specimens and reagent (gloves, lab vest, surgical mask, eye/face protection).

SYMBOL GLOSSARY

symbols as defined in ISO 15223			
	catalogue number		batch code
	use-by date		manufacturer
	keep away from (sun)light		temperature limit
	consult instructions for use		

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TECHNICAL SUPPORT

URL: www.inactivblue.com
email: info@inactivblue.com

MANUFACTURER INFORMATION

InActiv Blue bv
8730 Beernem (Belgium)
Tel: +32 50 791805
Fax: +32 50 791799