A&A BIOTECHNOLOGY
innovating life science

Manual

StayRNA™

Buffer for RNA protection in biological samples.

catalog #	size
208-100	100 ml
208-250	250 ml
208-500	500 ml

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Description

StayRNA™ is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA in situ in unfrozen specimens.

Tissue pieces are harvested and immediately submerged in StayRNA™ for storage without affecting the quality and quantity of RNA.

Thereby StayRNA™ eliminates the need to immediately process tissue specimens or to freeze samples in liquid nitrogen for later processing.

In case of a visible sediment heat StayRNA™ to 37 °C before use.

StayRNA™ stabilizes and protects RNA samples:

- 1 day / 24 hours at 37 °C
- 1 week at 25 °C
- 1 month at temp. from +2 °C up to +8 °C
- long-term storage at -20 °C or -80 °C

NOTE:

The above data are indicative only.

When selecting the storage conditions for samples intended for RNA isolation, the lowest possible / achievable temperatures and the shortest possible storage times at temperatures above -20 ° C should always be used.

StayRNA™ has been tested on several tissues:

- vertebrate species, including brain, heart, kidney, spleen, liver, testis, skeletal muscle, fat, lung and thymus
- *E. coli* cells
- *Drosophila*
- tissue culture cells
- white blood cells
- some plant tissues

StayRNA™ is compatible with most RNA isolation methods and with RNA isolation kits produced by A&A Biotechnology:
Total RNA Mini (cat. # 031-25, 031-100)
Total RNA Midi (cat. # 032-20)
Total RNA Maxi (cat. # 033-10)
Total RNA Mini Plus (cat. # 036-25, 036-100)
Total RNA Mini Plus Concentrator (cat. # 036-25C, 036-100C)
Micro RNA (cat. # 035-25)
Micro RNA Concentrator (cat. # 035-25C)

Additional equipment and reagents

- 10 mM Tris buffer, pH 8.5 cat # K-Tris-50

Material preparation

White blood cells

1. White blood cells should be separated from red blood cells and serum.
2. Suspend the white blood cells in 5 volumes of StayRNA™ ("volume" is defined as the volume of the sediment).

   StayRNA™ is not recommended for preserving RNA in whole blood, plasma or serum. Because of their high protein content, these fluids will form an insoluble precipitate.
3. Store at the appropriate temperature - see section „Storage of samples” page 6.

Bacteria cells

1. Centrifuge 1.5-3 ml of bacterial culture for 2 min at 12 000 x g.
2. Discard the supernatant.
3. Gently dissolve the pellet in 0.5-1 ml of StayRNA™.

   StayRNA™ is bacteriostatic, however the cells remain intact.
   E.coli cells stored in StayRNA™ for 1 month at temp. from +2 °C to +8 °C are intact.

Yeast cells

1. Centrifuge yeast culture containing up to 3 x 10^8 cells for 2 min at 12 000 x g..
2. Discard the supernatant.
3. Gently dissolve the pellet in 0.5-1 ml of StayRNA™.

   Yeast cells can be stored in StayRNA™ up to 8 hours at temp. 25 °C or to 1 week at temp. from +2 °C to +8 °C.
   In case of long-term storage the yeast cells should be incubated for 1 hour in StayRNA™. Next centrifuge the cells for 5 min at 12 000 x g.
   Discard supernatant and immediately freeze the sample. Store at temp. from -20 °C.

Animal tissue

1. Cut 20-50 mg of fresh animal tissue samples to a maximum thickness.

2. Transfer to a vessel of appropriate volume.

   Small organs such as rat liver, kidney and spleen can be stored whole in StayRNA™.

3. Add 5 volumes of StayRNA™ ("volume" is defined as the volume of the tissue), e.g. add 0.5-1 ml of StayRNA™ to 100-200 µl of sample.

4. Store at the appropriate temperature - see section „Storage of samples" page 6.

Plant tissue

1. Cut fresh plant tissue to maximum thickness in any one dimension of 0.5 cm. It is recommended to grind by grinding or homogenization on ice.

2. Transfer samples to Eppendorf tubes.

   Plant tissues that have natural barriers to diffusion, such as waxy coatings on leaves. will require disruption to allow StayRNA™ access to the tissue.
   Any method of disruption that breaks up the waxy coating (e.g. dicing or physically tearing) is suitable.

3. Add 5 volumes of StayRNA™.

4. Store at the appropriate temperature - see section „Storage of samples" page 6.

Tissue culture cells

1. 1 x 10^6 of tissue culture cells centrifuge for 2 min at 3000 x g.

2. Discard supernatant and suspend the pellet in 5 volumes of stayRNA™ ("volume" is defined as the volume of the sediment), e.g. add 250-500 µl of StayRNA™ to 20-50 µl of sample.

   If necessary wash the cell with a Tris buffer (10 mM Tris buffer, pH 8.5) or an equivalent buffer to remove the culture medium (before using StayRNA™).

3. Store at the appropriate temperature - see section „Storage of samples" page 6.

Storage of samples

Storage at -80 °C and -20 °C (recommended for long-term storage)

Do not freeze samples suspended in StayRNA™ before their preparation.
Incubate samples at 2-8 °C overnight, then remove them from StayRNA™ before storage at -20 °C or -80 °C to prevent the formation of salt crystals.
For tissue culture cells, do not remove the StayRNA™, simply freeze the whole solution.

Samples can subsequently be thawed at room temperature and refrozen usually without affecting the amount or the integrity of the recoverable RNA.

Storage at temp. from +2 °C up to +8 °C

Samples can be stored up to 1 month usually without any experimental evidence of RNA degradation.
Partial degradation may occur with some types of samples (especially solid tissues).

Storage at room temperature (25 °C)

Samples can be stored up to 1 week usually without any experimental evidence of RNA degradation.

RNA from samples stored at 25 °C for two weeks appears slightly degraded (i.e., marginally acceptable for Northern analysis, but still of sufficient quantity for nuclease protection assay or RT-PCR analysis).

If ambient temperature is above 25 °C, incubate samples in StayRNA™ on ice for a few hours if possible before storing at ambient temperature.

Storage at 37 °C

Samples can be stored up to 24 hours usually without any experimental evidence of RNA degradation.
Partial degradation may occur with some types of samples (especially solid tissues).

Note: regardless of the type of sample, RNA is partially degraded after a 3 days incubation at 37 °C.

RNA isolation from material in w StayRNA™

Samples stored in StayRNA™ may become hard, rubbery textured, and may be more difficult to homogenize thoroughly than fresh tissues. Dicing the tissue into smaller pieces with a scalpel can expedite homogenization.

Tissues

Tissues that have been stored in StayRNA™ should be removed from the storage solution with sterile forceps and submerged in a RNA isolation lysis solution - Fenozol or Fenozol Plus from RNA isolation kits.

Tissue homogenization should be rapid once the tissue is in the lysis solution.

Cells

To the cells suspended in StayRNA™ add the same volume of icy Tris buffer (10 mM Tris buffer, pH 8.5) to reduce the density of the sample and gently mix.
Centrifuge for 3 min at 5000 x g and remove the supernatant.
Resuspend the precipitate in 1 ml of Tris buffer (10 mM Tris buffer, pH 8.5). Centrifuge for 3 min at 5000 x g and remove the supernatant.
Directly after centrifugation pellet cells should be suspended in a lysis solution. Follow the protocol of RNA isolation.

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