User manual - NucleoProtect® RNA

1 Components

1.1 Product content

	NucleoProtect® RNA			
REF	**740400.6**	**740400.50**	**740400.250**	**740400.500**
NucleoProtect® RNA Solution	6 mL	50 mL	250 mL	500 mL
User manual	1	1	1	1

It is strongly recommended to read the protocol details in this user manual when using **NucleoProtect® RNA** for the first time. All technical literature is available online at ***www.mn-net.com***.

1.2 Consumables and equipment to be supplied by user

Consumables:

- Reaction tubes with lids of appropriate size (typically 1.5 mL, 2 mL, or 5 mL)
- Disposable pipette tips

Equipment:

- Manual pipettes
- Scalpel, scissors, forceps for tissue preparation and handling
- Personal protection equipment (lab coat, gloves, goggles)

2 Product description

2.1 The basic principle

NucleoProtect® RNA is a nontoxic, aqueous RNA stabilization and protection solution for cells and tissues. The solution permeates submerged, unfrozen cell and tissue samples. The RNA within these samples is protected from degradation during subsequent storage at appropriate conditions. Treatment of cells / tissue with NucleoProtect® RNA solution eliminates the need to immediately process or freeze samples after harvest. Cell or tissue samples should be treated with NucleoProtect® RNA as soon after collection as possible to obtain best results.

2.2 Product specification

NucleoProtect® RNA is suitable for RNA stabilization in most types of cells and tissues. It may not be effective with samples that are inadequately or slowly infiltrated by the solution, such as waxy plant tissue, fatty tissues like adipose tissue (however, brain tissue is compatible), bone, and samples exceeding 5 mm in any one dimension.

After complete permeation of samples with NucleoProtect® RNA solution (cells: ≥ 1 h at 4 °C – 25 °C; tissue: ≥ 12 h at 4 °C – 25 °C), samples can be stored long-term at -20 °C or below, while RNA integrity is preserved within the sample. NucleoProtect® RNA solution also preserves RNA integrity in samples stored for up to one month at 4 °C, one week at room temperature (15 – 25 °C), or one day at 37 °C once stabilized.

NucleoProtect® RNA Solution is not recommended for cell / tissue samples that have been frozen prior to immersion in NucleoProtect® RNA Solution.

NucleoProtect® RNA Solution is not recommended for RNA stabilization in whole blood samples. However, RNA in white blood cells can be stabilized after separation of cells from whole blood. Isolated white blood cells can be treated according to the standard protocol for cells (see section 5.1.).

NucleoProtect® RNA was shown to effectively stabilize RNA in bacterial / yeast culture pellets (protocol section 5.1) and plant leaf samples (protocol section 5.2). However, effective stabilization of RNA may depend on the species and sample properties.

NucleoProtect® RNA effectively stabilizes DNA, which can be isolated from stabilized samples using standard procedures.

NucleoProtect® RNA Solution is compatible with most RNA isolation methods, including NucleoSpin® and NucleoMag® RNA isolation kits as well as the NucleoZOL RNA isolation reagent (see section 6.2 for ordering information).

NucleoProtect® RNA is intended for research purposes only.

2.3 Quality of RNA from cell and tissue samples

Quality and yield of RNA from cell and tissue samples strongly depend on sample quality, proper handling from harvest to treatment with NucleoProtect® RNA solution, storage time and temperature, as well as the subsequent RNA purification method.

For HeLa cells treated with NucleoProtect® RNA, no significant change in RNA integrity (RIN) was observed after RNA isolation from samples stored for 1 month at 4 °C or 1 week at 18 – 25 °C or 1 day at 37 °C.

For mouse tissue (e.g., liver, kidney, and spleen) treated with NucleoProtect® RNA, no significant change in RNA integrity (RIN) was observed after RNA isolation from samples stored for one month at 4 °C or one week at 18 – 25 °C when compared to RNA integrity of RNA isolated from fresh, untreated samples. Storage at 37 °C might cause a decrease of several RIN units, even if samples are immediately stored at 37 °C after immersion in NucleoProtect® RNA solution. A decrease in RIN with 37 °C storage can be minimized if complete permeation of the tissue by NucleoProtect® RNA solution is allowed for ≥ 12 hours at 4 – 25 °C prior to the 37 °C storage.

Freeze-thaw compatibility: Samples properly submerged in NucleoProtect® RNA solution and stored for ≥ 12 hours at 4 °C – 25 °C can subsequently be frozen at -20 °C. When subjected to 10 freeze-thaw cycles we observed only minimal losses in RIN for tissues (≤ 0.5 units; tested for mouse liver, kidney, and spleen) and no losses at all for cultured cells (tested for HeLa cells).

2.4 Handling of sample material

Avoid exposure to RNases at any time prior to stabilization. Sample material should be as fresh as possible. Do not freeze sample before treatment with NucleoProtect® RNA solution. To minimize RNase activity between sample harvest and treatment with NucleoProtect® RNA, keep sample on ice. Do not disrupt or homogenize the sample at this point.

2.5 Stability of RNA in the sample upon stabilization

Allow the NucleoProtect® RNA solution to completely permeate the sample before storing it (cells: ≥ 1 h at 4 °C – 25 °C, tissues: ≥ 12 h at 4 °C – 25 °C). Afterwards the samples can be stored and / or shipped at the following conditions:

Storage temperature	Storage time
-20 / -80 °C	long term
4 °C	one month
18 °C – 25 °C (RT)	one week
37 °C	< 24 h

2.6 Stability of RNA after isolation

After RNA isolation samples should always be kept on ice during work for optimal stability. Contamination with RNases (general lab ware, fingerprints, dust) may lead to degradation of isolated RNA. Freeze isolated RNA at -20 °C for short-term storage, and freeze isolated RNA at -80 °C for long-term storage.

3 Product storage conditions

Store NucleoProtect® RNA solution at room temperature (15 – 25 °C) until: see package label. Storage / transport at lower temperatures may cause precipitation of salt. If a precipitate is visible, heat the solution to 50 °C for 30 min while stirring it. After cooling down to room temperature the solution is ready to use again.

Note: Small amounts of residual precipitate at the bottom are not critical and do not affect product function.

4 Safety instructions

NucleoProtect® RNA does not contain hazardous substances or mixtures.

ATTENTION!

Biological samples used in conjunction with NucleoProtect® RNA might pose a potential biohazard risk if the sample contains infectious agents. Follow your local, state / provincial, and / or national regulations when working with such samples.

There is no data on inactivation of infectious agents by NucleoProtect® RNA. Biohazardous samples have to be treated accordingly, even after treatment with NucleoProtect® RNA solution! Biohazardous samples retrieved from NucleoProtect® RNA solution after storage still have to be considered and treated as such!

5 Protocols

Before starting the procedure:

- Make sure to use fresh sample material.
- Do not freeze samples before treatment with NucleoProtect® RNA solution.
- To minimize RNase activity between sample harvest and treatment with NucleoProtect® RNA, keep sample in a tube on wet ice (0 °C). However, it is recommended to immediately treat the samples with NucleoProtect® RNA after harvesting.

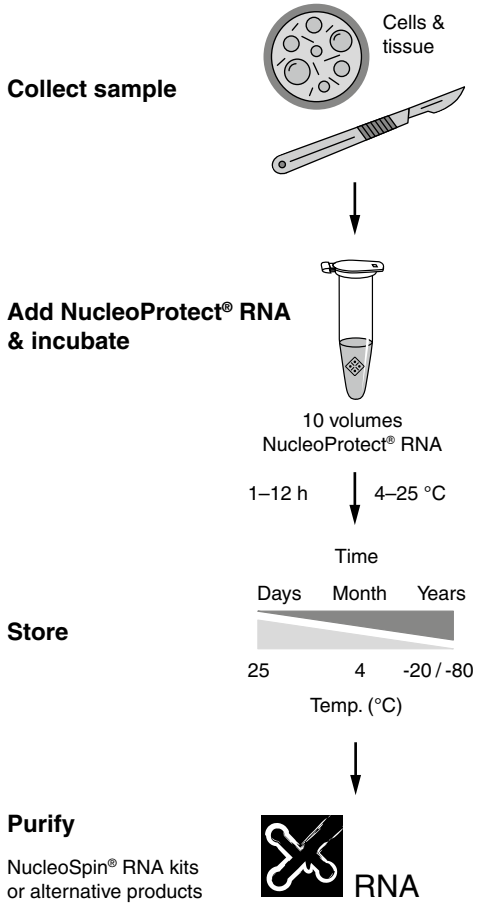

Figure 1 Schematic overview on the use of NucleoProtect® RNA solution

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5.1 Protocol for the stabilization of RNA in cell samples

Pellet cells from the culture medium or buffer solution (e.g., by centrifugation) and remove excess supernatant from the pellet.

Note: Cell samples may be washed with PBS or an equivalent low protein buffer in order to remove cell culture medium.

Optional: Resuspend the cell pellet in a small volume of PBS in order to loosen up the cells.

Add 10 volumes of NucleoProtect® RNA reagent. For cell pellets with a volume smaller than 100 µL, simply add 1 mL NucleoProtect® RNA reagent.

Moderately agitate the tube by inverting it several times to suspend the cells in the solution. Do not vortex strongly!

Store resuspended cells for ≥ 1 h at 4 °C – 25 °C to allow complete permeation with NucleoProtect® RNA reagent. If you observe cell clumps or incomplete resuspension store for ≥ 12 hours at 4 °C – 25 °C to allow complete permeation.

Store stabilized cell samples at 18 – 25 °C for up to 1 week or at 4 °C for up to one month or at ≤ -20 °C for long-term storage (see also section 2.5 for appropriate storage conditions).

Note: NucleoProtect® RNA does neither lyse nor disrupt cells. However, cells or cell pellets might slightly shrink during storage (depending on cell type and water content).

Note: If the optional resuspension step of the cell pellet is omitted, the cell pellet shall not exceed 5 mm in any one dimension.

5.2 Protocol for the stabilization of RNA in tissue samples

Dissect tissue into pieces with a maximum diameter of 5 mm in any one dimension. Place the tissue sample in an appropriate tube (e.g., a 2 mL tube for one 5 × 5 x 5 mm sample).

Add 1 mL of NucleoProtect® RNA reagent onto the sample (or at least 10 volumes of reagent relative to the sample). Manually agitate the tube (e.g., by inverting) in order to loosen the sample from the tube wall. The sample must be completely submerged in the stabilization solution and must have been in contact with the solution from all sides.

Store for ≥ 12 hours at 4 °C – 25 °C to allow complete permeation with NucleoProtect® RNA reagent.

Store stabilized tissue samples at 18 – 25 °C for up to 1 week at 4 °C for up to one month, or at ≤ -20 °C for long-term storage (see also section 2.5 for appropriate storage conditions).

Note: NucleoProtect® RNA does does not lyse or disrupt tissue samples. However, tissue samples might shrink slightly during storage (depending on tissue type and water content).

Note: Small organs, such as mouse kidney, liver, and spleen, and other tissue samples of < 5 mm size in any one dimension can be submerged whole in NucleoProtect® RNA Solution.

5.3 Protocol for the isolation of RNA from NucleoProtect® RNA treated samples

Take samples stabilized in NucleoProtect® RNA out of the refrigerator / freezer and allow them to come to room temperature.

- **Cells**
  Centrifuge the sample in order to pellet the cells. Remove the supernatant by pipetting. Proceed with the lysis step of your preferred RNA isolation method.
- **Tissue**
  Retrieve the sample from the NucleoProtect® RNA solution with sterile forceps. Remove excess NucleoProtect® RNA solution by blotting the sample onto a paper towel. At this point the tissue may be further dissected to obtain pieces of desired or adequate size for subsequent RNA isolation. This can be done at room temperature. Proceed with the lysis step of your preferred RNA isolation method.

Make sure the sample amount complies with the specifications of the RNA purification method / kit used! We recommend to use RNA purification products from MACHEREY-NAGEL (see section 6.2). Appropriate alternative products may be used as well.

Note: Tissue samples stored in NucleoProtect® RNA typically shrink slightly due to loss of water from the sample and may harden. Thus, homogenization of the tissue in the lysis step of the RNA isolation procedure might require stronger mechanical disruption for complete lysis as compared to fresh tissue.

Note: Typically, a precipitate will be visible after storage at -20 °C or below. Tissue samples can be retrieved from the stabilization solution and used for RNA isolation protocols according to section 5.3 without any further actions. However, precipitates in cell samples should be redissolved (e.g., by pipetting up and down). Pellet cells by centrifugation and proceed with removal of the supernatant according to section 5.3.

6 Appendix

6.1 Troubleshooting

Degraded RNA

- *Quality of sample material*
  Make sure to use only freshly harvested sample material.
- *Frozen sample material*
  NucleoProtect® RNA solution is not recommended for immersion of previously frozen sample material. Use fresh and unfrozen sample material only.
- *Sample too large*
  Make sure the sample does not exceed 5 mm in any dimension.
- *Inappropriate sample/solution ratio*
  Make sure to use 10 volumes of NucleoProtect® RNA solution relative to the sample (volume or mass).
- *Exceeded storage time*
  Make sure not to exceed maximal recommend storage time for a given storage temperature. See section 2.5. for appropriate storage conditions.
- *Incomplete permeation*
  Allow the NucleoProtect® RNA solution to completely permeate the sample before freezing it (cells: ≥ 1 hour at 4 °C – 25 °C; tissue: ≥ 12 hours at 4 °C – 25 °C)
- *Inappropriate storage vial or volume*
  Make sure, that the sample is well surrounded by the NucleoProtect® RNA solution. The tissue piece should be “floating” freely in the reagent and be in contact with the solution from all sides.
- *RNA degradation after purification*
  Make sure to follow recommendations in section 2.5.

No RNA

- *Incompatible isolation procedure*
  Make sure, that the RNA isolation procedure is compatible with NucleoProtect® RNA Solution. For recommendations see section 6.2 or contact our technical support (tech-bio@mn-net.com).
- *Solution carryover*
  Make sure to remove excess NucleoProtect® RNA solution from the sample before starting the RNA isolation procedure from the sample.

6.2 Ordering information

Product	REF	Pack of
NucleoProtect® RNA	740400.50 / 250 / 500	50 / 250 / 500 mL
NucleoSpin® RNA Plus	740984.10 / 50 / 250	10 / 50 / 250 preps
NucleoSpin® RNA Plus XS	740990.10 / 50 / 250	10 / 50 / 250 preps
NucleoSpin® RNA	740955.10 / 50 / 250	10 / 50 / 250 preps
NucleoSpin® RNA XS	740902.10 / 50 / 250	10 / 50 / 250 preps
NucleoSpin® RNA Midi	740962.20	20 preps
NucleoSpin® 96 RNA	740709.2 / 4 / 24	2 / 4 / 24 × 96 preps
NucleoSpin® 8 RNA	740698 / .5	12 / 60 × 8 preps
NucleoMag® RNA	744350.1 / 4	2 / 4 × 96 preps
NucleoSpin® RNA / Protein	740933.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® TriPrep	740966.10 / .50 / .250	10 / 50 / 250 preps
NucleoZOL	740404.200	200 mL
rDNase Set	740963	1 set
Collection Tubes (2 mL)	740600	1000 pieces

Visit ***www.mn-net.com*** for more detailed product information or contact our technical support (tech-bio@mn-net.com)

6.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

This MACHEREY-NAGEL product is carrying documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. The provided warranty is limited to the data specifications and descriptions as given in the original MACHEREY-NAGEL literature. No other statements or representations, written or oral, by MACHEREY-NAGEL’s employees, agents or representatives, except written statements signed by a duly authorized officer of MACHEREY-NAGEL are authorized. They should not be relied upon by the costumer and are not a part of a contract of sale or of this warranty.

Liability for all possible damages that occur in any connection with our products is limited to the utmost minimum as stated in the general business terms and conditions of MACHEREY-NAGEL in their latest edition which can be taken from the company’s website. MACHEREY-NAGEL does not assume any further warranty.

Products and their application are subject to change. Therefore, please contact our Technical Service Team for the latest information on MACHEREY-NAGEL products. You may also contact your local distributor for general scientific information. Descriptions in MACHEREY-NAGEL literature are provided for informational purposes only.

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