SileksMagNA™ Plasma100 DNA/RNA Isolation kit

Cat. #: KRPS100

SileksMagNA™ Plasma1ML circulating nucleic acid Isolation kit

Cat. #: KRPS1ML

Technical Support

Purpose of kits: isolation of circulating nucleic acid nucleic acids (DNA and RNA)

Storage conditions: +4°C

Transportation conditions: no special conditions required

Contents of kits

Each kit contains reagents sufficient for 100 isolation procedures. One isolation procedure contemplates using of 100 µL (KRPS100) or 1 mL (KRPS1ML) of starting material (plasma or serum).

Kit Components	Volume	
	KRPS100	KRPS1ML
• **START** Buffer	12 mL	120 mL
• **Lysis&Binding** Buffer	24 mL	240 mL
• **SileksMagNA™** Magnetic Particles	1 mL	1 mL
• **Wash 1** Buffer	30 mL	30 mL
• **Wash 2** Buffer	30 mL	30 mL
• **Wash 3** Buffer	30 mL	30 mL
• **Final Wash** Buffer	30 mL	30 mL
• **Elution** Buffer	10 mL	10 mL
• **RNA Protector**	0.5 mL	0.5 mL

Contents:

1. Safety and precautions

Some of the kit components can cause severe health damage if swallowed, breathed and in case of contact with eyes or skin.

Avoid mixing the kit components with strong acids and alkali, chloric disinfectants and bleachers. It can cause reactions with production of toxic fumes. If you use an aspirator to remove wash buffers from the test tubes, check that its collector is empty and does not contain any components, listed above. In the kits, some organic compounds find use, inhalation exposure of which can cause vertigo and headaches.

Avoid contact of the kit components with eyes, skin and clothing.
In case of contact with eyes, rinse immediately with plenty amounts of water, for at least 15 minutes. Make the rinsing more thorough by separating the eyelids with fingers. If discomfort symptoms (redness, irritation and burning) persist, seek medical attention. In case of contact with skin, immediately wash the skin with soap and plenty amounts of water.

2. Description

The kits are developed for easy, efficient and fast (~30 min) isolation of free circulating nucleic acid (mRNA, exosomal RNA and DNA, viral DNA and RNA) from serum or plasma. Isolated nucleic acids are suitable for PCR or other molecular biology applications (labeling, cloning, sequencing, reverse transcription etc). The kit is based on reversible binding of nucleic acids to the surface of magnetic particles. The isolation protocol can be scaled for isolation of larger amounts of nucleic acids.

Most of DNA and RNA in an organism are located inside its cells. But few nucleic acids can be found free circulating in the bloodstream. This kind of DNA and RNA molecules, called cNA (circulating nucleic acids), originate mostly from dead and destructed cells. Some cNA molecules are synthesized by living cells and excreted into the blood as a part of intercellular signaling system. Existence of such circulating nucleic acids in blood allows for development of minimally invasive diagnostic approaches for screening of various clinical diseases and disorders. Discovering of specific circulating DNA and/or RNA create a unique opportunity for early detection of different pathological processes. For instance, early stages of cancer and many viral infections can be detected by this method. Noninvasive prenatal screening during pregnancy period or diagnostic of some forms of diabetes can be done this way as well.

Depending on the task, different amounts of starting material can be used - from 100 µl to 10 mL of plasma or serum.

Terminology:

Blood plasma is a liquid component of whole blood. It does not contain cells, but contains all proteins (globulins, albumins, fibrinogens etc.), electrolytes, clotting factors and other extracellular components of blood. The simplest way to obtain plasma from whole blood is to centrifuge it at 2,000–3,000×g for 5 minutes.
In schematic form it can be described as: **Plasma = Blood – Cells**

Blood serum is a blood plasma without clotting factors and other proteins involved in this process. Usually it is a liquid that remains after blood coagulation (clotting).
In schematic form it can be described as: **Serum = Plasma – Clotting Factors**

The blood level of free DNA, produced from dead cells and microorganisms, is usually low. The main source of free DNA in blood is apoptosis. This process is typical in particular for oncological diseases.

The principle of method used in the kits is based on a reversible adsorption of nucleic acids on the surface of magnetic particles. A schematic procedure of isolation of nucleic acids from blood is shown in Figure 1. After the sample is lysed and nucleic acids contained in it are bound to the magnetic particles, they must be washed with buffers from the kit. After the wash cycles are finished, the pellet of magnetic particles must be dried and after it nucleic acids can be eluted from the particles.

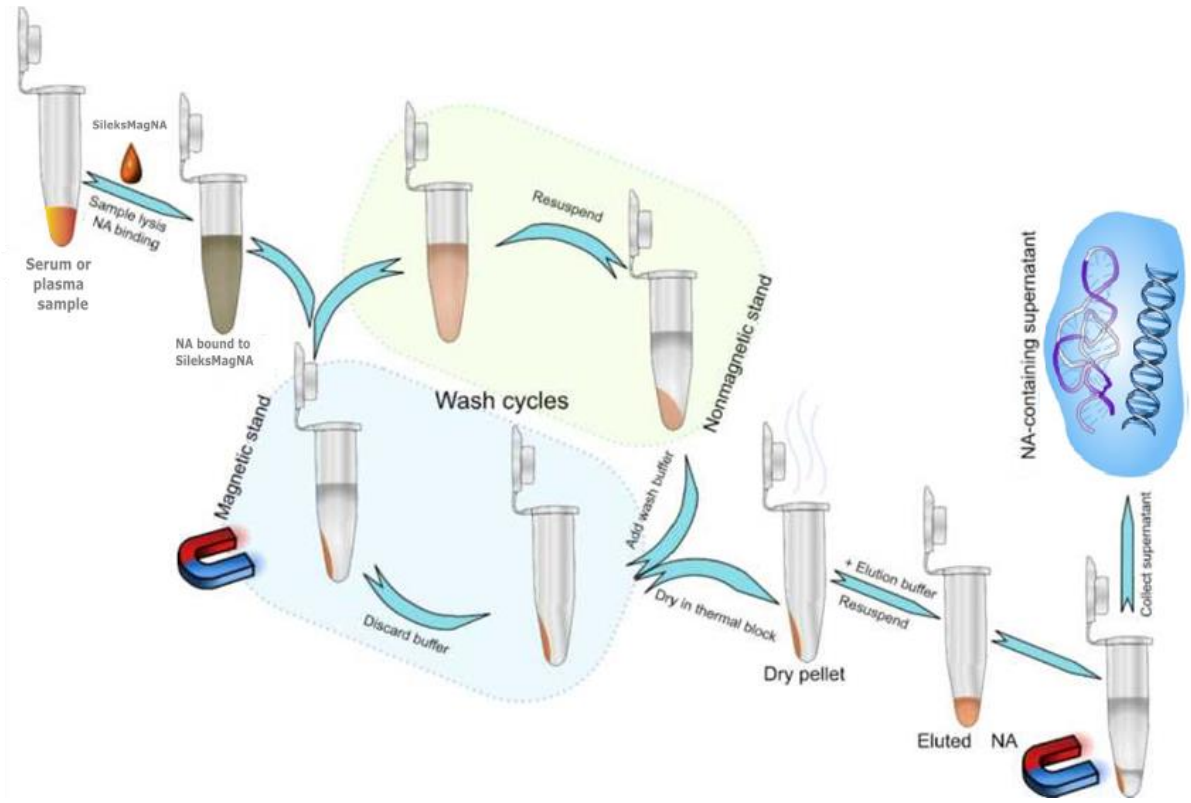


Figure 1. DNA/RNA isolation procedure

3. Preparing plasma from the whole blood

Correct plasma preparation is a key point, determining success of the whole isolation procedure.

Blood cells are damaged and destroyed during transportation and storage. It results in release into plasma of additional pool of nucleic acids, not related to target cNA. Presence of nucleic acids from blood cells, damaged during transportation, decreases reliability and sensitivity of target cNA detection.

To protect blood cells from damage during transportation and storage we recommend using for blood collection only special tubes. Such tubes contain chemical stabilizers like K_3EDTA, formaldehyde and other agents, which increase of cell stability.

The most reliable way to obtain high quality plasma sample is to separate plasma from cells immediately after blood sample collection. The following storage requires temperature not higher than +4°C. For long-term storage plasma sample can be frozen at -80°C or -60°C.

We recommend the following plasma separation procedure:

1. Centrifuge the blood sample at room temperature for 20 minutes at 400 x g. Such soft centrifugation is required to avoid damage of blood cells.

2. Carefully transfer the plasma to a fresh tube. Avoid capturing cells at the interphase zone when collecting plasma.

3. Centrifuge collected plasma at room temperature for 10 minutes at 5000 x g. This procedure removes remaining few blood cells.

4. Transfer the plasma to a fresh tube. Store the sample at temperatures not exceeding +4°C.

Recommended time for storage of plasma:

+4°C - up to 2 weeks
-20°C - up to 1 month
-80°C or -60°C - several years

4. Isolation protocol

Read before starting your work!
Please read the Comments section carefully before proceeding.

Check all buffers for precipitates. Precipitate formation has no permanent effect on the buffer quality. If a precipitate has been formed, warm the buffer at 50°C until it becomes clear.

Lisys&Binding, Wash 1, Wash 2 buffers must be well shaked before use to produce a uniform suspension. Statement "*thoroughly mixed buffer*" hereinafter means that the buffer must be shaken 5-10 times.

Statement "*mix thoroughly*" hereinafter means that the solution must be mixed in one of the following ways:

- by manual pipetting (at least 15 pipettings of every sample are required for good resuspension);
- using compact mixer (for example, LabMix Mixer 201) (5 seconds on low or medium speed).

DNA/RNA (circulating nucleic acids) isolation from 1 mL of plasma or serum

Lysis

1. Add **1 mL** of plasma or serum to a 15 mL tube.
2. Add **1.2 mL** of well mixed **START** buffer and mix thoroughly by pipetting
3. Incubate at room temperature for **5 minutes**.
   Longer incubation (up to 15 minutes) allows to increase DNA/RNA yield.
4. In a separate clean tube mix the following components: **2.4 mL** of well mixed **Lysis&Binding** buffer and **7 µL** of well mixed magnetic particles **SileksMagNA**. Mix thoroughly.
5. Add the prepared suspension of magnetic particles to the tube containing prepared sample. Mix thoroughly. Incubate for **5 minutes** at room temperature**,** mix tube content several times during incubation.
6. Place the tube in a **magnetic rack** to collect the particles (it could take up to 1 minute). Discard the supernatant. Be careful not to disturb magnetic particles collected at the tube wall.

Wash 1

7. Place the tube in a **non-magnetic rack**. Resuspend magnetic particles in **300 µL** of well mixed **Wash 1** buffer and mix thoroughly until a homogeneous suspension is obtained. **Transfer obtained suspension** in 1.5 mL tube for further isolation. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

   The use of shaker improves the result and shortens the procedure.
   If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
8. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Wash 2

9. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 2** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

   The use of shaker improves the result and shortens the procedure.
   If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
10. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Wash 3

11. Place the tube in a **non-magnetic rack**. Add **300 µL** of **Wash 3** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

    The use of shaker improves the result and shortens the procedure.
    If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
12. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Final Wash

13. Place the tube in a **non-magnetic rack**. Add **300 µL** of **Final Wash** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

    The use of shaker improves the result and shortens the procedure.
    If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.

Important note! At this stage (with particles in Final Wash buffer) DNA/RNA bound to magnetic particles can be stored for very long time without degradation. Temperature of storage may vary in very wide range: from room temperature (+22°C) to -70°C. Lower temperature provides better preservation. After the storage period is over, the protocol can be continued from step 14.

14. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
15. Incubate the tube in a **thermal block at 60°C** for **5 minutes** to dry the pellet of magnetic particles..

Elution

16. Add **50 µL** of **Elution** buffer. Thoroughly resuspend particles until a homogeneous suspension is obtained.
    If you wish to have higher DNA/RNA concentration you can use 25 µL of Elution buffer instead of 50 µL.
17. Incubate in a **thermal block at 60°C** for **5 minutes**.
18. Place the tube in a **magnetic rack** to collect the particles. Transfer the DNA/RNA-containing supernatant into a new tube.
19. Add to collected DNA/RNA-containing supernatant **5 µL** of **RNA Protector** ($^1/_{10}$ from the volume of **Elution** buffer added).

Store the collected solution of isolated DNA/RNA at -20 °C.
If your task is to isolate RNA, we recommend adding RNA Protector (see Appendix 2) or RNasin immediately after the isolation procedure is complete. Reverse transcription must also be started as soon as possible. RNA may degrade relatively fast, decreasing the sensitivity of its detection.

DNA/RNA (circulating nucleic acids) isolation from 100 µL of plasma or serum

Lysis

1. Add **100 µL** of plasma or serum to a 15 mL tube.
2. Add **120 µL** of well mixed **START** buffer and mix thoroughly by pipetting
3. Incubate at room temperature for **5 minutes**.
   Longer incubation (up to 15 minutes) allows to increase DNA/RNA yield.
4. In a separate clean tube mix the following components: **240 µL** of well mixed **Lysis&Binding** buffer and **5 µL** of well mixed **SileksMagNA™** magnetic particles. Mix thoroughly.
5. Add the prepared suspension of magnetic particles to the tube containing prepared sample. Mix thoroughly. Incubate for **5 minutes** at room temperature**,** mix tube content several times during incubation.
6. Place the tube in a **magnetic rack** to collect the particles (it could take up to **1 minute**). Discard the supernatant. Be careful not to disturb magnetic particles collected at the tube wall.

Wash 1

7. Place the tube in a **non-magnetic rack**. Resuspend magnetic particles in **300 µL** of well mixed **Wash 1** buffer and mix thoroughly until a homogeneous suspension is obtained. We recommend for the best results to **transfer obtained suspension** in 1.5 mL tube for further isolation.
   Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

   The use of shaker improves the result and shortens the procedure.
   If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
8. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Wash 2

9. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 2** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

   The use of shaker improves the result and shortens the procedure.
   If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
10. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Wash 3

11. Place the tube in a **non-magnetic rack**. Add **300 µL** of **Wash 3** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

    The use of shaker improves the result and shortens the procedure.
    If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.
12. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Final Wash

13. Place the tube in a **non-magnetic rack**. Add **300 µL** of **Final Wash** buffer and mix thoroughly until a homogeneous suspension is obtained. Incubate for **3 minutes** at room temperature. Mix the contents of the tube several times during incubation.

    The use of shaker improves the result and shortens the procedure.
    If using a shaker, incubate thoroughly homogenized particles for 2 minutes at 1250-1500 rpm depending on shaker model.

Important note! At this stage (with particles in Final Wash buffer) DNA/RNA bound to magnetic particles can be stored for very long time without degradation. Temperature of storage may vary in very wide range: from room temperature (+22°C) to -70°C. Lower temperature provides better preservation. After the storage period is over, the protocol can be continued from step 14.

14. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
15. Incubate the tube in a **thermal block at 60°C** for **5 minutes** to dry the pellet of magnetic particles..
16. Add **50 µL** of **Elution** buffer. Thoroughly resuspend particles until a homogeneous suspension is obtained.
    If you wish to have higher DNA/RNA concentration you can use 25 µL of Elution buffer instead of 50 µL.

Elution

17. Incubate in a **thermal block at 60°C** for **5 minutes**.
18. Place the tube in a **magnetic rack** to collect the particles. Transfer the DNA/RNA-containing supernatant into a new tube.

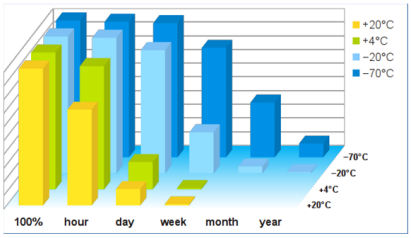
19 Add to collected DNA/RNA-containing supernatant **5 µL** of **RNA Protector** ($^1/_{10}$ from the volume of **Elution** buffer added).

Store the collected solution of isolated DNA/RNA at -20 °C.
If your aim is to isolate RNA, we recommend adding RNA Protector (see Appendix 2) or RNasin immediately after the isolation procedure is complete. Reverse transcription must also be started as soon as possible. RNA may degrade relatively fast, decreasing the sensitivity of its detection.

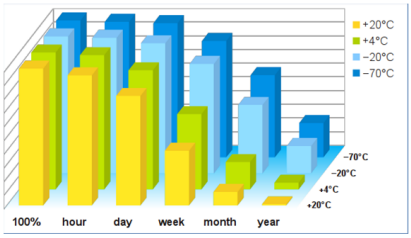
Appendix 1

Long-term storage of nucleic acids in FinalWash buffer

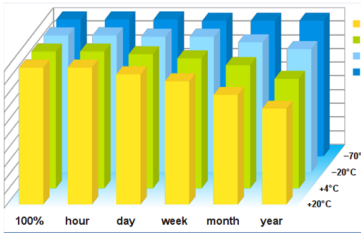
The diagrams show DNA/RNA stability during storage at different conditions:



MilliQ Water



Water with *RNasin* added



Bound to magnetic particles in *FinalWash* buffer

Our experimental data shows that storage of nucleic acids bound to magnetic particles in *FinalWash* buffer is the most effective method of long-term protection from degradation.

Appendix 2

RNA Protector

RNA Protector is a synthetic non-protein substance for protecting RNA preparations.

It stabilizes RNA in enzymatic reactions and protects it from RNase degradation and oxidative damage. Due to its non-protein nature it does not lose its activity after freezing of heating, as in case of standard RNase inhibitors of protein nature.

We recommend using ***RNA Protector*** primarily with the following of our kits:

- SileksMagNA™ Plasma100 DNA/RNA Isolation kit and SileksMagNA™ Plasma1ML DNA/RNA Isolation kit (cat.#: KRPS100 and KRPS1ML),
- SileksMagNA™ FFPE DNA/RNA Isolation kit (cat.#: KRFF100),
- SileksMagNA-G™ Cell Culture DNA Isolation kit and SileksMagNA™ Cell Culture DNA/RNA Isolation kit (cat.#: KDCC100 and KRCC100),

We also recommend using ***RNA Protector*** in any case where RNA is object of further investigation.

You should add ***RNA Protector*** immediately in the sample collected after elution.

The recommended amount of ***RNA Protector*** is $^{1}/_{10}$ from volume of eluent.

For example, if 50 µL of **Elution** buffer was used, after elution and collection of the sample it is necessary to add 5 µL of ***RNA Protector***.

The mode of action of ***RNA Protector*** is different from that of protein RNase inhibitors. For this reason we recommend using ***RNA Protector*** only for previously purified RNA preparations. You should not use ***RNA Protector*** for tasks requiring specific inhibition of RNases or analogous enzymes.

RNA Protector is perfect for the cases when, after obtaining of RNA-containing preparation, additional procedures are required, including the long incubation at elevated temperature (for example, DNase treatment for removing of DNA traces following by inactivation of enzyme).

5. Recommendations for protocol modifications

1. The isolation procedure described in protocol above can be used with minor modifications for isolation of cNA from 100 µL up to 10 mL of blood serum or plasma.

   For isolation from sample with different initial quantity that mentioned in protocol, you should follow the ratio below:

   n mL of serum or plasma + 1.2 x n mL **START** buffer + 2.4 x n mL **Lysis&Binding** buffer

   where n - amount of serum or plasma, taken as initial sample.

   Do not try to use smaller amount of **Lysis&Binding** buffer than in given ratio. It will result in worsening of sample quality, and, consequently, reducing of PCR sensitivity.

2. We recommend using the following amounts of magnetic particles:

from 100 µL to 1 mL of initial sample	5 µL
from 1 mL to 10 mL of initial sample	10 µL

   Nevertheless, some optimization of amount of magnetic particles may be required depending on the quality of the sample for isolation and aims of the research.
   For such optimization, we recommend to increase volume of magnetic particles in increments of 2.5 µL starting from recommended above, but not more than two-fold

3. During isolation from any amount of start material из you should use not less than 200 µL of wash buffers. Recommended amount is 300 µL of each of wash buffers.
   Nevertheless, some optimization of amount of wash buffers may be required depending on the quality of the sample for isolation and aims of the research.
   For such optimization, we recommend to increase volume of wash buffers in increments of 100 µL, but no more than 600 µL.

4. Elution reaches its maximal efficiency when the volume of Elution buffer added is three-fold or more relative to the start volume of suspension of magnetic particles. Minimal volume of Elution buffer required must not be smaller than two-fold relative to initial amount of magnetic particles.

5. For assessment of isolation quality we recommend PCR-based methods rather than spectrophotometry and other optical detection methods. Proper normalization is required, especially for intercomparison of concentrations of nucleic acids, isolated with these kits and with kits from other manufactures. Impact of admixtures and co-precipitants, contained in kits from other manufacturers may be a source of wrong estimation of isolation efficiency.

IMPORTANT NOTE

The quality of suspending of particles is a key moment to achieve good isolation results.

For obtaining DNA/RNA with high yield and quality, it is necessary to resuspend particles as thoroughly as possible at each washing step.

6. Comments

General notes

1 mL of healthy human plasma contains, on average, 1 to 50 ng of circulating nucleic acids (cNA). Amount of cNA increases in human plasma in case of disease, in particular, in oncological patients, and during pregnancy.
Because of low concentration of obtained cNA, there is no sense in using spectrophotometric detection methods for assessment of isolation quality, even with the use of intercalating fluorescent dyes. Quantitative PCR is the only reliable way of this assessment.
During construction of primers and probes it is necessary to take into consideration strong fragmentation of cNA, amplicon size, if possible, should be no more than 100 bp.
During investigation of RNA, we do not recommend to use oligo(dT) primers for first strand cDNA synthesis. Using of random primers allows for increasing the method sensitivity. Some researchers prefer to use gene-specific primers, however it can decrease the method sensitivity.

Comments for protocol steps

1. Before using of plasma or serum, make sure that it does not contain blood cells. If you suspect the presence of blood cells in plasma, repeat the procedure of preparing plasma (see paragraph Preparing plasma from the whole blood).
   When frozen plasma or serum is used, after thawing of sample, it is necessary to mix plasma or serum especially thoroughly until full uniformity.

4. Magnetic Particles **SileksMagNA™** must be mixed with **Lysis&Binding** buffer before use. Adding of particles separately, before or after addition of **Lysis&Binding** buffer, reduces isolation efficiency.
   For regular work you can mix particles and **Lysis&Binding** buffer in advance and store as suspension at +4°C. It is necessary to mix particles in buffer thoroughly before use.

5. For maximal sorption particles should be evenly distributed across the whole volume. Take care that the particles do not settle down during incubation process. If this takes place, mix the tube content until the suspension looks homogenous.

13. Long-time storage of isolated nucleic acids (DNA and RNA) on magnetic particles in **Final Wash** buffer allows accumulation of samples, by isolating them till this step once available, followed by synchronous final isolation. Such an approach allows avoiding of possible damage of isolated nucleic acids during storage.

17. During elution, incubation at 60°C for more than 5 minutes results in reduction of purity of isolated nucleic acids because of elution of impurities, partially absorbed on particles.

19. Immediately after collection of DNA/RNA-containing supernatant, add **RNA Protector**. This reagent stabilizes RNA during the storage and enzymatic reactions. For more reliable maintaining of RNA you can also further add RNase inhibitor.

20. To reaction mixture for first strand cDNA synthesis add obtained eluate in amount no more than 1/8 of final volume of reaction mixture. The recommended amount for reaction is 1/10 of volume. For example, for first strand cDNA synthesis in 25 µL, recommended amount of eluate is 2.5 µL. Using of larger amount of eluate can result in reaction inhibition.

Storage of DNA/RNA, that already is rather fragmented, results in even more strong fragmentation, up to full degradation. So, we do recommend using of nucleic acid for further work immediately after isolation.

7. Related products

1. **SileksMagNA™** Magnetic particles, 50 mg/mL, 1 mL, Cat. #: MPR001

   SileksMagNA™ Magnetic particles, 50 mg/mL, 10 mL, Cat. #: MPR010

2. **LabMix Mini 201** handheld mixer, Cat. #: EQMM201

   LabMix Mini 201 allows to achieve as thorough mixing as it is with manual pipetting. It also increases results reproducibility.

3. **Magnetic racks for working with magnetic particles**

   MagRack6, Cat. #: EQRM06
   MagRack16, Cat. #: EQRM16
   MagRack40, Cat. #: EQRM40
   MagRack50ML, Cat. #: EQRM50ML

8. Contact information

We will always be happy to help you.

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