SileksMagNA™ FFPE DNA/RNA Isolation kit

Cat. No KRFF100

Technical Support

Purpose of kit: isolation of nucleic acids (DNA and RNA) from formalin-fixed, paraffin-embedded (FFPE) samples

Storage conditions: +4°C

Transportation conditions: no special conditions required

Kit Contents

Provided Reagents are sufficient for:

100 isolation procedures of either genomic DNA or total RNA

50 simultaneous isolation procedures of genomic DNA and total RNA

Kit Components	Volume
• **Proteinase**	1 mL
• **Proteinase Buffer** (x1-fold)	10 mL
• **START** Buffer	12 mL
• **Lysis&Binding** Buffer	24 mL
• **SileksMagNA™** Magnetic Particles	1 mL
• **Wash 1** Buffer	30 mL
• **Wash 2** Buffer	30 mL
• **Wash 3** Buffer	30 mL
• **Final Wash** Buffer	30 mL
• **Elution** Buffer	10 mL
• **RNA Protector**	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

2.1. Introduction

The kit is developed for easy, efficient and fast (~30 min) isolation of nucleic acids (DNA and RNA) from formalin-fixed, paraffin-embedded (FFPE) samples.

The kit provides reagents for 100 isolation procedures of either genomic DNA or total RNA. You can also simultaneously isolate both genomic DNA and total RNA from single sample. Simultaneous isolation represents two separate isolation procedures with common start stages, so provided reagents are sufficient for 50 simultaneous isolation procedures of genomic DNA and total RNA.

The peculiarity of isolation of nucleic acids from FFPE samples and subsequent work with isolated material is that during the fixation and embedding operations, nucleic acids (DNA and RNA) in FFPE samples are severely fragmented and chemically modified by formaldehyde. Therefore, nucleic acids isolated from FFPE samples often are pretty much fragmented and have critical distinction from ones obtained from fresh or frozen samples. The treatment with formaldehyde affects not only the isolation procedure of nucleic acids itself, but also subsequent procedures involving enzymatic reactions. The formaldehyde modifications and traces of formaldehyde have a strong inhibitory effect on many routinely used enzymes including thermostable polymerases used in polymerase chain reaction (PCR).

So, it is often impossible to estimate the quality of RNA, isolated from FFPE samples, by gel electrophoresis. 18S and 28S rRNA bands often have low visibility, they are stretched and smeared or, in the worst case, may be completely absent.

Depending on the quality of the sample, size of the nucleic acids can be highly variable. Average size of RNA, isolated from FFPE samples, is approximately 100 nucleotides. The size of DNA can be much larger, sometimes up to 500 nucleotides.

Sample quality and integrity of nucleic acids are determined by different factors:

- *type of tissue,*
- *storage time of the sample,*
- *conditions for fixation and embedding (pH of the used buffers, the temperature, the quality of the reagents),*
- *storage conditions of the sample.*

The proposed procedure of isolation of nucleic acids considerably reduces the number of cross-links, formed during the formaldehyde fixation.
However, the process of fixation and embedding irreversibly damages nucleic acid so that it cannot be used for the same applications as a nucleic acid of the standard quality.
Some applications (polymerase chain reaction (PCR), and first strand cDNA synthesis) may require modifications to allow the use of degraded nucleic acids, isolated from FFPE samples.

Important !!!

We recommend to modify your PCR system so that the resulting product (amplicon) would have a length of no more than 100 bp. For cDNA synthesis we do not recommend to use oligo(dT) primers. 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.

2.2. Overview of the Method

The kit is based on the well-optimized procedure designed by our company for isolation of nucleic acids using a reversible sorption on magnetic particles. The isolation procedure from FFPE samples includes additional step of proteinase treatment. But in contrast to standard procedure of the treatment with proteinase, that require a long incubation - overnight or longer - we use a specially designed buffers that allow reducing proteinase incubation time, to accelerate the isolation and to increase the yield of nucleic acids.

First, the paraffin should be removed from FFPE sample. Here are a variety of methods that can be used for deparaffinization. These methods are listed in section Deparaffinization. Then the sample is treated with proteinase according to one of the protocols (Isolation of total RNA, Isolation of genomic DNA, Simultaneous isolation of total RNA and genomic DNA from the single tissue sample). After the treatment with proteinase the mixture is centrifuged to remove the insoluble components. The supernatant, containing the nucleic acids, is transferred to a fresh tube. The buffer, that improves the purification, is added to the supernatant. After a short incubation, magnetic particles are added. The nucleic acid is adsorbed on magnetic particles. The supernatant is removed. Then a number of washing procedures is used to remove impurities. Pure nucleic acid is eluted in 30 to 100 µl of Elution buffer, depending on the subsequent tasks.
We recommend using the isolated nucleic acid immediately for further procedures.
For long-term storage of the purified nucleic acid follow the recommendations in protocol.

3. Preparing the Tissue Samples

The fixation and embedding procedures always result in fragmentation and crosslinking of nucleic acids in the sample. To minimize the damage of RNA and DNA we recommend:

- fixate tissue sample in formalin as quickly as possible after surgical removal
- fixate tissue sample in temperature range of +4 to +10ºC and no longer than 24 hours, longer fixation time critically increases the damage of nucleic acids
- use low-melting paraffin, because the use of high-melting paraffin and high temperatures during embedding increase the damage and fragmentation of nucleic acids
- store FFPE samples in temperature range of 0 to +10ºC, storage at room temperature increase the degradation of nucleic acids

Immediately before the procedure, it is necessary to cut samples for isolation into 10 µm sections. Isolation from thicker sections impairs the quality of isolation and results in lower yield of nucleic acids. The section surface area should be 0.5-1.5 cm^2. Usually only a single section is enough for isolation. We strongly recommend to start the isolation with one section. In exceptional cases, when the section contains low amount of material, or sample was stored longer than 10-20 years, you could try to use two or three sections.

Place the section in 1.5 mL tube. Close the tube and store it at 0 to +10ºC until the isolation begins.

4. Deparaffinization

4.1. Standard protocol using xylene

Reagents for deparaffiniztion are not supplied with the kit and are purchased additionally.
Ethanol-containing solutions are not supplied with the kit and must be prepared by customer.

Deparaffinization using xylene is standard and the most common procedure.

1. Add **1 mL** of **xylene** to the tube, containing a section.
   Vortex for **10 seconds**. Incubate at room temperature for **10 minutes**. Vortex the tube during the incubation at least 2 more times.
   Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
   Carefully remove the supernatant.
   Attention! The tissue on the bottom of the tube is transparent and difficult to see. Do not remove it accidentally while removing the supernatant.
2. Add **1 mL** of xylene once more and repeat the incubation and centrifugation. Carefully remove the supernatant.
   Attention! The tissue on the bottom of the tube is transparent and difficult to see. Do not remove it accidentally while removing the supernatant.
3. Add **1 mL** of **96% ethanol**.
   Vortex for **10 seconds**.
   Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
   Carefully remove the supernatant.
4. Add **1 mL** of **90% ethanol**.
   Vortex for **10 seconds**.
   Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
   Carefully remove the supernatant.
5. Add **1 mL** of **70% ethanol**.
   Vortex for **10 seconds**.

Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
Carefully remove the supernatant.

6. Keep the tube cap open for **10 minutes** to dry the pellet.

Proceed to the appropriate protocol selection 5.1, 5.2 or 5.3.

4.2. Deparaffinization using heptane and methanol

Reagents for deparaffiniztion are not supplied with the kit and are purchased additionally.
Ethanol-containing solutions are not supplied with the kit and must be prepared by customer.

Deparaffinization using heptane and methanol is one of the most effective. This procedure results in the more compact pellet and isolation of nucleic acids gives reasonable good results.

1. Add **500 µL** of **heptane** to the tube, containing a section.
   Vortex for **10 seconds**. Incubate at room temperature for **10 minutes**. Vortex the tube during the incubation at least 2 more times.
2. Add **25 µL** of **methanol**.
   Vortex for **10 seconds**.
   Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
   Carefully remove the supernatant.
3. Add **1 mL** of **96%** ethanol.
   Vortex for **10 seconds**.
   Centrifuge the tube at **12,000-18,000×g** for **2 minutes**.
   Carefully remove the supernatant.
4. Keep the tube cap open for **10 minutes** to dry the pellet.

Proceed to the appropriate protocol selection 5.1, 5.2 or 5.3.

5. Isolation protocols

Please read before starting your work!
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 mixed 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).

5.1. Isolation of total RNA

Extraction

1. Add **100 µL** of **Proteinase Buffer** and **10 µL** of **Proteinase** to the tube, containing a sample after deparaffinization.
   Vortex the tube and place in the thermal block at **+60ºC**.
   Incubate the tube for at least 1 hour. Depending on the tissue material, the sample may be dissolved not completely. This does not affect the result.
2. Place the tube in the thermal block at **+80ºC**.
   Incubate for **30 minutes**. Maximal incubation time could be **60 minutes**. Longer incubation time leads to more severe degradation of RNA.
3. Cool down the tube **on ice** for **2 minutes**.
4. Centrifuge the tube at **12.000-18.000×g** for **10 minutes**.
5. Transfer the RNA-containing supernatant to a fresh tube without disturbing the pellet.

Lysis

6. Add **120 µL START** buffer and mix thoroughly by pipetting.
7. Incubate at room temperature for **5 minutes**.
   Longer incubation (up to 15 minutes) allows to increase RNA yield.

Binding

8. In a separate fresh tube mix the following components: **240 µL** of well mixed **Lysis&Binding** buffer and **5 µL** of well mixed magnetic particles **SileksMagNA**. Mix this blend thoroughly.
9. Add the prepared suspension of magnetic particles to the tube containing lysed sample. Mix thoroughly. Incubate at room temperature for **5 minutes**. Mix tube content two or three times during incubation.
10. 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

11. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 1** buffer and mix thoroughly until a homogeneous suspension is obtained.

Wash 2

12. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
13. 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.

Wash 3

14. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
15. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 3** buffer and mix thoroughly until a homogeneous suspension is obtained.
16. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
17. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **FinalWash** buffer and mix thoroughly until a homogeneous suspension is obtained.

FinalWash

Important note! At this stage (with particles in **Final Wash** buffer) RNA bound to magnetic particles can be stored for a very long time. Temperature of storage may vary in very wide range: from room temperature (+20°C) to +4°C, –20°C and –70°C. Lower temperature provides better preservation. After the storage period is over, the protocol can be continued directly from next step.

18. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
19. Incubate the tube (with its cap open) in a **thermal block** to dry particles at **60°C** for **5 minutes**.

Elution

20. Add **30 µL** of **Elution** buffer. Mix the particles thoroughly until a homogeneous suspension is obtained. *Volume of the Elution buffer can be increased if necessary up to 100 µL.*
21. Incubate the tube in a **thermal block** at **60°C** for **5 minutes**.
22. Place the tube in a **magnetic rack** to collect the particles. Transfer the RNA-containing supernatant to a fresh tube. Add **3 µL of RNA Protector**.

RNA is very labile and difficult to store. It is necessary to perform reverse transcription as soon as possible. RNA can quickly degrade during the storage. It leads to a strong decrease of the general sensitivity of the detection method. Reagent *RNA Protector* protects RNA, but does not completely preserve it from degradation. Its volume must be one tenth from the volume of *Elution* buffer. If elution is in larger volume than recommended, you may need to purchase additional amount of *RNA Protector* (in this case, please, contact us for further information).
The resulting total RNA may contain traces of DNA. But it is not a problem when the amplification system is properly developed. However, if there is a need to have RNA free of the traces of DNA, we recommend using the treatment of isolated RNA with DNase. After this treatment you can inactivate or remove DNase by one of the following methods:

- Reprecipitate RNA in the presence of a co-precipipant (e.g., glycogen). Considering the strong fragmentation of RNA, the losses could be greater than 50%.
- Purify using magnetic particles ("SileksMagNA™ Reaction Products purification kit").
- Remove DNase using an appropriate sorbent for enzyme removing.
- Inactivate DNase by heating at 70°C.

5.2. Isolation of DNA

Extraction

1. Add **100 µL** of **Proteinase Buffer** and **10 µL** of **Proteinase** to the tube, containing a sample after deparaffinization.
   Vortex the tube and place in the thermal block at **+60ºC**.
   Incubate the tube for at least 1 hour. Depending on the tissue material, the sample may be dissolved not completely. This does not affect the result.
2. Place the tube in the thermal block at **+90ºC**. Incubate for **2 hours**.
3. Cool down the tube **on ice** for **2 minutes**.
4. Centrifuge the tube at **12.000-18.000×g** for **10 minutes**.
5. Transfer the DNA-containing supernatant to a fresh tube without disturbing the pellet.
6. Add **120 µL START** buffer and mix thoroughly by pipetting.

Lysis

7. Incubate at room temperature for **5 minutes**.
   Longer incubation (up to 15 minutes) allows to increase DNA yield.

Bi

8. In a separate fresh tube mix the following components: **240 µL** of well mixed **Lysis&Binding** buffer and **5**

µL of well mixed magnetic particles **SileksMagNA**. Mix this blend thoroughly.

9. Add the prepared suspension of magnetic particles to the tube containing lysed sample. Mix thoroughly. Incubate at room temperature for **5 minutes**. Mix tube content two or three times during incubation.
10. 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

11. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 1** buffer and mix thoroughly until a homogeneous suspension is obtained.

Wash 2

12. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
13. 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.

Wash 3

14. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
15. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **Wash 3** buffer and mix thoroughly until a homogeneous suspension is obtained.
16. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

FinalWash

17. Place the tube in a **non-magnetic rack**. Add **300 µL** of well mixed **FinalWash** buffer and mix thoroughly until a homogeneous suspension is obtained.

Important note! At this stage (with particles in **Final Wash** buffer) DNA bound to magnetic particles can be stored for a very long time. Temperature of storage may vary in very wide range: from room temperature (+20°C) to +4°C, –20°C and –70°C. Lower temperature provides better preservation. After the storage period is over, the protocol can be continued directly from next step.

18. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
19. Incubate the tube (with its cap open) in a **thermal block** to dry particles at **60°C** for **5 minutes**.

Elution

20. Add **30** µL of **Elution** buffer. Mix the particles thoroughly until a homogeneous suspension is obtained. *Volume of the Elution buffer can be increased if necessary up to 100 µL.*
21. Incubate the tube in a **thermal block** at **60°C** for **5 minutes**.
22. Place the tube in a **magnetic rack** to collect the particles. Transfer the DNA-containing supernatant to a fresh tube.

Store isolated DNA at -20 °C. The resulting genomic DNA may contain traces of RNA. Usually it is not a problem. However, if there is a need to have DNA free of RNA traces, we recommend using the treatment of isolated DNA with RNase. After this treatment you can inactivate or remove RNase by one of the following methods:
Precipitate DNA in the presence of a co-precipipant (e.g., glycogen). Considering the strong fragmentation of DNA, the losses could be greater than 50%.
Purify using magnetic particles ("SileksMagNA™ Reaction Products purification kit").
Remove RNase using *an appropriate sorbent for enzyme removing*.

5.3. Simultaneous isolation of total RNA and genomic DNA from the single tissue sample

1. Add **100 µL** of **Proteinase Buffer** and **10 µL** of **Proteinase** to the tube, containing a sample after deparaffinization.
   Vortex the tube and place in the thermal block at **+60ºC**.
   Incubate the tube for at least **1 hour**. Depending on the tissue material, the sample may be dissolved not completely. This does not affect the result..
2. Cool down the tube **on ice** for **2 minutes**.
3. Centrifuge the tube at **12.000-18.000×g** for **10 minutes**.
4. Transfer the RNA-containing supernatant to a fresh tube without disturbing the DNA-containing pellet.
   Attention!!! DNA-containing pellet can be stored in the following conditions:

room temperature	1-2 hours
+2 to +10°C	1 day
-20°C	1 week to 1 month

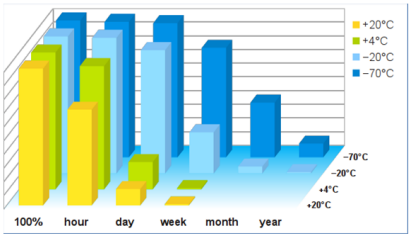
5. Incubate the tube with RNA-containing supernatant at **+80ºC** for **15 minutes**.
   Longer incubation time leads to more severe degradation of RNA.
6. Proceed to step ***6*** of protocol **5.1. Isolation of total RNA.**

7. Add **100 µL** of **Proteinase Buffer** and **10 µL** of **Proteinase** to the tube with the DNA-containing pellet. Vortex the tube and place in the thermal block at **+60ºC**. Incubate the tube for at least **1 hour**. Depending on the tissue material, the sample may be dissolved not completely. This does not affect the result.
8. Place the tube in the thermal block at **+90°C**. Incubate for **2 hours**.
   IPMORTANT! Do not vortex the tube or mix the tube content during incubation!
9. Centrifuge the tube at **12.000-18.000×g** for **10 minutes**.
10. Transfer the DNA-containing supernatant to a fresh tube without disturbing the pellet.
11. Proceed to step **6** of protocol **5.2. Isolation of DNA**.

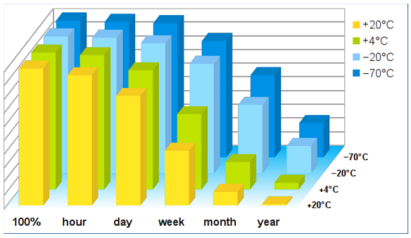
6. Appendices

Appendix 1
Long-term storage of nucleic acids in *FinalWash* buffer

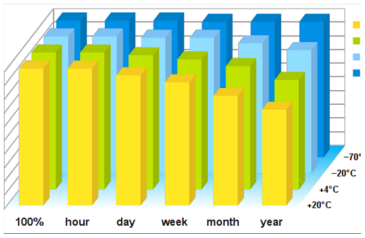
The diagrams show RNA stability during storage at different conditions:



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).

7. Recommendations for protocol modifications

1. For a first isolation, we recommend using one 10 µm section. If the amount of isolated RNA/DNA is too low, you can increase the amount of sections stepwise up to five. it should be remembered that increasing of amount of starting material decreases the purity of isolated RNA/DNA.

2. We recommend using 5 µL of magnetic particles:
   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 50 µL, but no more than 600 µL.

4. To increase DNA/RNA concentration in the final sample you can decrease the added amount of Elution buffer. 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.

8. Comments

General notes
Because of low concentration of obtained nucleic acids, 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 RNA/DNA, 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. Deparaffinization procedure is not critical for subsequent isolation of RNA/DNA. For removing of paraffin you can use xylene, toluene and other organic solvents, effectively diluting paraffines. More important procedure is the treatment with aqueous ethanol, because such a treatment allows for rehydration of dehydrated tissue, thereby improving of further RNA/DNA extraction.

2. For effective RNA isolation the complete degradation of tissue by proteinase is not necessary. In some instances, heating of deparaffinized sample in Proteinase Buffer without addition of Proteinase itself allows for obtaining the same amount of RNA, but with higher purity relative to RNA after Proteinase treatment. For DNA isolation, proteinase treatment is necessary and important.

3. Cooling down on ice after Proteinase treatment and following thermal treatments improves the pellet formation.

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.

6. 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.

7. 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.

8. 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.

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 RNA/DNA, 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.

9. 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

10. Contact information

We will always be happy to help you.

Phone: +49 7632-82-31964

e-mail: info@sileks.de