SileksMagNA-G™ Swabs DNA Isolation kit
Cat. #: KDSW100

SileksMagNA™ Swabs DNA/RNA Isolation kit
Cat. #: KRSW100

Technical Support

Purpose of kits: isolation of 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.

Kit Components	Volume
• **START** Buffer	12 mL
• **Lysis&Binding** Buffer	24 mL
• **SileksMagNA-G™** Magnetic Particles (for Cat. #: KDSW100)	1 mL
• **SileksMagNA™** Magnetic Particles (for Cat. #: KRSW100)	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** (for Cat. #: KRSW100)	0.5 mL

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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 kit is designed for efficient and fast (~ 40 min) isolation of DNA and RNA from epithelial swabs / scrapes (buccal, vaginal, cervical, urethral, etc.).
The isolated DNA and RNA can be used both in PCR and for any other molecular biological applications (labeling, cloning, sequencing, etc.).
The kit contains all the necessary reagents and buffers. The isolation protocol can be modified to scale up when more material is required. For scaling it is necessary to proportionally change the amount of used reagents.
The principle of the method used in the kit is based on the reversible binding of nucleic acids on the surface of magnetic particles.
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 is shown in Figure 1. The general scheme of treatment resolves itself into lysis of cells in the sample and releasing of contained in them nucleic acids, which bind thereafter on magnetic particles. Bound nucleic acids undergo number of washes with buffers from the kits. After the wash cycles are finished, the pellet of magnetic particles must be dried and after that nucleic acids (DNA and RNA) can be eluted from the particles.

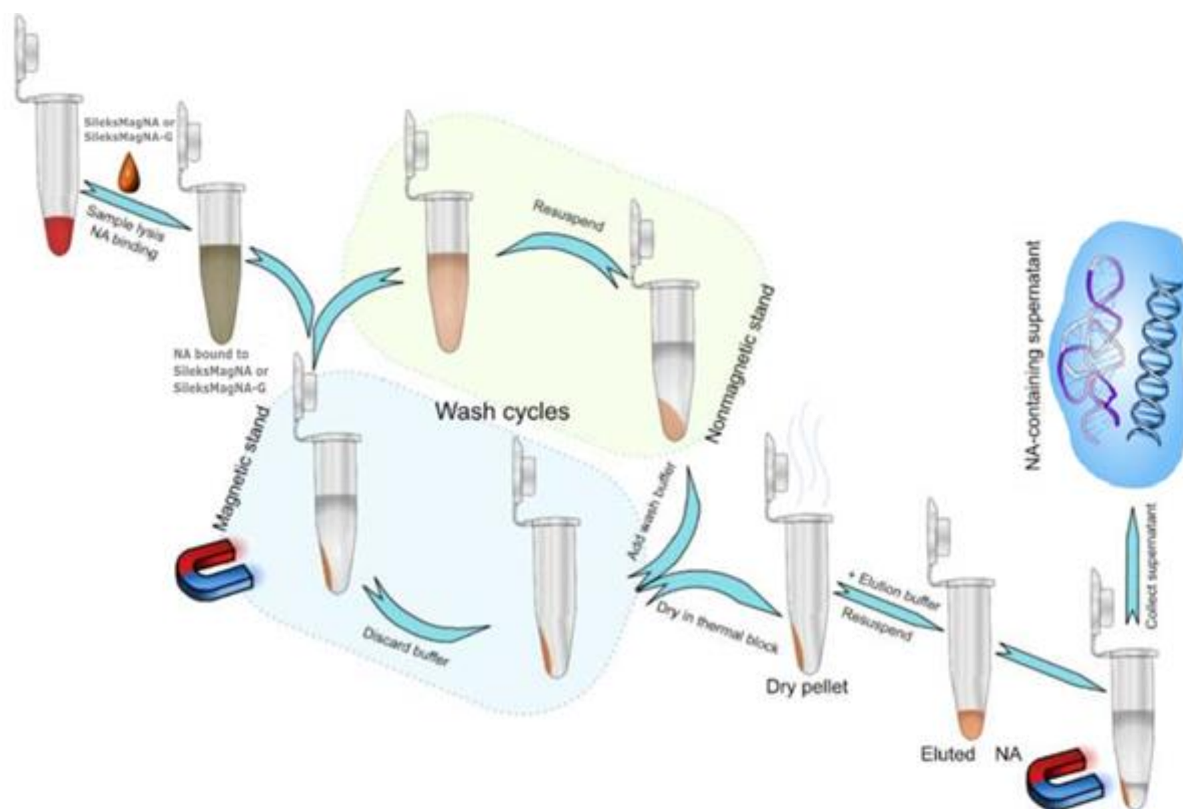


Figure 1. DNA/RNA isolation procedure

The kit is optimized for the isolation of nucleic acids from approximately 10^5 cells. But in every isolation, optimization may be required depending on the sample used. The main recommendation is to select the minimum number of cells needed to obtain a consistent and repeatable result. It usually ranges from $0.5x10^5$ to $5x10^5$ cells per sample.

Depending on the type of magnetic particles used, it is possible to preferentially isolate DNA (SileksMagNA-G) or DNA and RNA (SileksMagNA). But in any case, both types of nucleic acids will always be present in the resulting preparation. This is very useful, for example, when studying expression. The presence of genomic DNA allows the obtained expression levels to be normalized to the number of cells in the sample. Because the amount of DNA in the probe is directly proportional to the number of cells taken for isolation. And RNA after the reaction of reverse transcription and synthesis of the first strand of cDNA makes it possible to determine the actual levels of expression. Then, using certain formulas using data on the DNA content, it is possible to calculate with high accuracy the relative expression levels in different samples, even if they contain a very different number of cells.

3. 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).

Taking a sample of an epithelial swab or scrapes

Self-taking a swabs or scrapes from the inside of the cheek (buccal).

Taking other types of swabs / scrapes requires medical assistance.

Sample preparation

1. Add **500 µL** of saline (0.9% sodium chloride solution) into a clean 1.5 ml tube.
2. Collect epithelial cells from the inside of the cheek with a sterile cotton swab by gently rolling the swab over the cheek for approximately 20 seconds.
3. Insert the swab with the collected cells into the tube with saline solution, rotate the swab with rotary movements for 30 seconds.
4. Remove the swab by squeezing it against the wall of the tube. For further work, go to item **6** of this protocol.

Recommendations for manipulation with a sample of an epithelial swab / scrapes (buccal, vaginal, cervical, urethral, etc.) taken in a medical laboratory in a container for collecting samples.

5. If the swab or scraping brush has not been removed from the tube, discard the drops from the tube lid by brief centrifugation to avoid splatter when opening the tube. Remove the swab by squeezing it against the wall of the tube. For further work, go to item **6** of this protocol.

6. Centrifuge the tube at **5,000 rpm** for **3 minutes**. Carefully discard the supernatant to avoid touching the cell pellet.
   Add **300 µL** of saline, resuspend the cells to remove mucus residues.
   Repeat centrifugation at **5,000 rpm** for **3 minutes**. Carefully discard the supernatant to avoid touching the cell pellet.
7. Resuspend cells in **100 µL** saline.
8. Add **120 µL** of well mixed **START** buffer and mix thoroughly by pipetting

Lysis

9. Incubate at room temperature for **5 minutes**.
   Longer incubation (up to 15 minutes) allows to increase DNA/RNA yield.
10. In a separate clean tube mix the following components: **240 µL** of well mixed **Lysis&Binding** buffer and **7 µL** of well mixed magnetic particles (**SileksMagNA-G** for DNA isolation or **SileksMagNA** for DNA/RNA isolation). Mix thoroughly.
11. 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.
12. 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

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

Wash 2

15. 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.
16. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.

Wash 3

17. 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.

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

Final Wash

19. 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.

Elution

20. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
21. Incubate the tube in a **thermal block at 60 °C** for **5 minutes** to dry the pellet of magnetic particles.
22. Add **100 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 50 µL of Elution buffer instead of 100 µL.
23. Incubate in a **thermal block at 60 °C** for **5 minutes**.
24. Place the tube in a **magnetic rack** to collect the particles. Transfer the DNA/RNA-containing supernatant into a new tube.
25. If the purpose of isolation was RNA, immediately after collecting the supernatant containing DNA and RNA, add **RNA Protector** reagent to the sample in amount of one tenth ($^{1}/_{10}$) of the collected volume. This drug stabilizes RNA during storage and subsequent enzymatic reactions.

    Store the collected solution of isolated DNA/RNA at -20 °C.

4. Troubleshooting

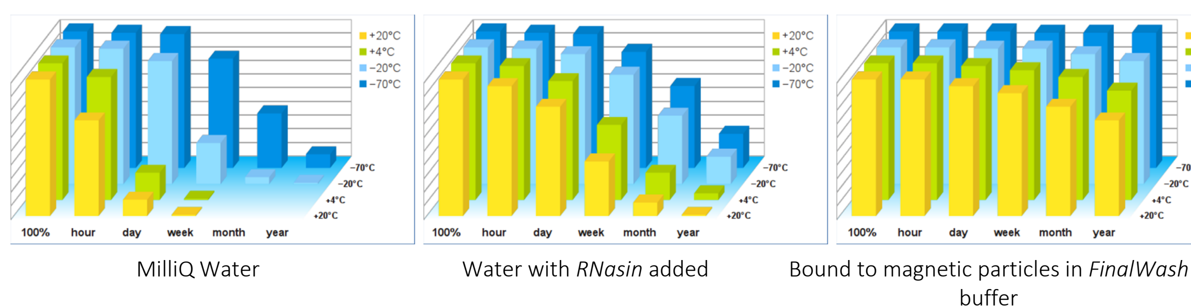
Purity of isolated nucleic acid usually is determined by the ratio of optical densities at wavelength 260 and 280 nm. The OD_{260}/OD_{280} ratio must be in the range of 1.7 - 2.0. For assessment of small amounts of nucleic acid you can use fluorimetric detection and PCR-based methods.

Problem	Possible causes	Solution
Low nucleic acid yield	**A.** Sample condition	
	1. Not enough nucleic acids-containing cells were collected.	1. Use more source material or elute DNA in a smaller volume of *Elution* buffer..
	2. The collected material was stored for a long period of time or had undergone several freeze–thaw cycles.	2. Repeat materials collection procedure.
	B. Particles were not dried completely before adding *Elution* buffer,	Increase the drying time after removing *Final Wash* buffer.
	C. Incomplete lysis	Mix the sample more thorough after adding *START* buffer..
	D. Too big volume of *Elution* buffer added	Find optimal buffer volume to achieve the required concentration of nucleic acids.
The OD_{260}/OD_{280} ratio is too low	**A.** Protein contamination	1. Try to achieve better particles resuspension on each step of the isolation procedure. 2. Use smaller amount of particles or bigger volume of *Elution* buffer.
	B. Incomplete removal of wash buffer components, primarily *FinalWash*	1. Check the temperature in the thermostat ensures complete drying of the particles, adjust the time and temperature to ensure that the particles is completely dry. 2. Some magnetic racks collect magnetic particles into a compact point, which will impair the drying process of the particles. Select a rack where the particles are distributed more evenly on the tube wall.
The isolated nucleic acids seem to be degraded	The sample material is too old or had undergone several freeze–thaw cycles.	Repeat materials collection procedure. Avoid freezing samples during the transportation and storage period.

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 non-proteinaceous substance designed to protect RNA preparations.

This reagent stabilizes RNA in enzymatic reactions and protects RNA from the action of RNases and from oxidative damage. Since this reagent is non-proteinaceous in nature, it does not lose activity upon freezing and heating, as is the case with standard protein ribonuclease inhibitors.

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

- Isolation of DNA / RNA from blood plasma and serum on magnetic particles SileksMagNA (cat. #: KIRPS100),
- Isolation of circulating nucleic acid from blood plasma and serum on magnetic particles SileksMagNA (cat. #: KIRCNA1ML),
- Isolation of DNA / RNA from formalin-fixed paraffinized (FFPE) samples (cat. #: KIRFFPE100),
- Isolation of DNA / RNA from cell cultures (cat. #: KIRCC100),

We also recommend using the **RNA Protector** in all cases where RNA will be the subject of further research.

RNA Protector should be immediately added to the collected sample after elution.

The recommended amount of **RNA Protector** is $^{1}/_{10}$ of the collected volume after elution.

For example, if 50 µl of elution buffer was used for elution, 5 µl of **RNA Protecto**r should be added after elution.

The principle of action of **RNA Protector** differs from the principle of action of protein ribonuclease inhibitors. Therefore, we recommend using **RNA Protector** only for pre-purified RNA preparations. The **RNA Protector** should not be used for applications that require targeted inhibition of ribonucleases or other similar enzymes.

RNA Protector is ideal in cases where additional procedures are required after receiving a preparation containing RNA, including prolonged incubation at an elevated temperature (for example, treatment with DNases to remove traces of DNA with subsequent inactivation of the enzyme).

5. Recommendations for protocol modifications

1. The isolation procedure described in protocol above can be used with minor modifications for isolation of DNA/RNA from a different number of cells.
   For isolation from a larger number of cells, it is necessary to resuspend the cells before isolation in a larger volume of saline.

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

   n µL of blood + 1.2 x n µL **START** buffer + 2.4 x n µL **Lysis&Binding** buffer

   where n – amount of blood (µL), 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. 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.

3. 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.

4. 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

The quantity of DNA or DNA/RNA isolated depends on a quantity of nucleic acids-containing cells.
The theoretical particle capacity is 10 µg of total nucleic acid per 10 µL of magnetic particles.
To provide the most complete isolation of total nucleic acid (DNA and all types of RNA), it is necessary to use the correct ratio.

Comments for protocol steps

10. Magnetic Particles (**SileksMagNA** or **SileksMagNA-G**) 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.

11. 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.

13-19. After thorough resuspension in wash buffers, the particles need to incubate for 3-5 minutes in manual isolation and 1-2 minutes using a shaker at 1250-1500 rpm, depending on the particular shaker model.

19. 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.

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

It is necessary to avoid freeze–thaw cycles during storage of isolated nucleic acids because they result in nucleic acid fragmentation, up to complete degradation. That's why we recommend using of nucleic acids for further work immediately after isolation

When storing the isolated nucleic acid, freeze-thaw should be avoided, as this leads to fragmentation, up to complete destruction. Therefore, we strongly recommend using nucleic acid for further work immediately after its isolation.

If it is necessary to obtain exclusively DNA or RNA, we recommend, after isolation, to carry out additional processing of the sample with the appropriate enzyme - DNase or RNase - followed by mandatory re-precipitation of the remaining nucleic acid.
For such a reprecipitation, we recommend using our kit "Purification of DNA / RNA after enzymatic reactions", cat. number KREN100.

Working with RNA, observe the following rules:
add the resulting eluate to the synthesis reaction of the first chain in an amount of not more than 1/8 of the volume of the final volume of the reaction mixture.
The recommended amount taken into the reaction is 1/10 of the volume.
For example, when final volume of the reaction mix is 25 µL, the recommended amount of eluate must be no more than 3 µL. The use of more eluate can lead to inhibition of the reaction.

7. Related products

1. **SileksMagNA-G™** Magnetic particles, 50 mg/mL, 1 mL, Cat. #: MPD001

   SileksMagNA-G™ Magnetic particles, 50 mg/mL, 10 mL, Cat. #: MPD010

   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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