SileksMagNA-Direct™ circulating Nucleic Acid (DNA / RNA) Isolation Kit (isolation from plasma, serum, urine and other biological fluids)

Cat.No: KIRCN2ML

Application:	Isolation of circulating Nucleic Acids (DNA / RNA)
Storage:	+4ºC
Transportation conditions:	no special conditions required
Number of isolations:	100 isolations from 2 ml of the original sample If necessary isolate from larger sample volume the buffer **Lysis&Denaturation** must be additionally purchased. cat. No: BLD250, 250 mL
Usage of isolated material:	first strand synthesis, PCR, sequencing

PLEASE NOTE! Due to the properties of particles chemistry and the isolation procedure, obtained nucleic acids can not be used for restriction analysis, as well as for visualization using electrophoresis.

Kit Content

Kit contains all essential components for isolation procedure.

Components	Volume
• **Lysis&Denaturation SileksMagNA-Direct™** Buffer	250 mL
• **SileksMagNA-Direct™** magnetic particles	1 mL
• **Wash SileksMagNA-Direct™** Buffer	30 mL
• **Elution SileksMagNA-Direct™** Buffer	10 mL
• **Fixation SileksMagNA-Direct™** Buffer	0.5 mL

Table 1. Description and properties of magnetic particles SileksMagNA-Direct™

Core	Iron oxide encapsulated in an inert shell
Type of magnetization	Superparamagnetic (no residual magnetization)
Particle shape	Sphere
Size	150-200 nm
Concetration	50 mg/mL
Particle capacity	4 µg total nucleic acids per 10 µL of particles 8 µg total nucleic acids per 1 mg of particles
Storage buffer	ddH_2O
Storage conditions	+4 °C, do not freeze

Content:

1. Precautions

Some components of the kit may be harmful to health if swallowed, inhaled, in contact with skin or eyes.

Do not mix kit components with acids, strong alkalis, chlorine disinfectants and bleaches. This can lead to reactions with the release of toxic gases. If you use an aspirator to remove Wash buffers, make sure the trap flask is empty or does not contain the components mentioned above.

Avoid getting kit items on clothing, skin, and eyes.

In case of contact with eyes, rinse immediately with plenty of water, continuing this procedure for at least 15 minutes. The effectiveness of washing increases if you forcefully pull off the eyelids with your fingers. At preservation of unpleasant symptoms (reddening, irritability, burning sensation) it is obligatory to ask for medical help. In case of contact with skin, wash immediately with soap and plenty of water..

2. Description

SileksMagNA-Direct™ particles are a fundamentally new type of particles. The surface of the particles is coated with a special polymer that provides selective binding of nucleic acids of all shapes and sizes. Protein binding on the surface of particles is minimal. As a rule, proteins bind in a complex with nucleic acids and are removed during the washing procedure.

In contrast to silica-coated particles, *SileksMagNA-Direct*™ particles do not require special conditions for binding nucleic acids, such as the presence of salts in the required concentration, alcohols, buffers with a certain pH value.

SileksMagNA-Direct™ particles effectively bind nucleic acids in single or double-stranded form, as well as short oligonucleotides. Binding can take place in any conditions - in the presence or absence of salts, chaotropic agents, alcohols, ionic and non-ionic detergents, in distilled water.

SileksMagNA-Direct™ particles were developed for the isolation of free circulating nucleic acids (cNA) from plasma, serum, urine, and other biological samples. The use of particles for isolation from other source is in the process. As soon as new results will be available, we will propose new protocols and related reagents.

In contrast to silica-coated particles, *SileksMagNA-Direct*™ particles firmly bind and hold the bound nucleic acid. Nucleic acid can be desorbed from the surface of the particles only using a special *Elution* buffer for *SileksMagNA-Direct*™. After isolation it is necessary add to collected eluate the *Fixation* buffer for *SileksMagNA-Direct*™, which stabilizes the obtained nucleic acid and protects it from damage.

SileksMagNA-Direct ™ isolation procedure is the following:

1. **sample preparation** (lysis and denaturation): release of nucleic acids from complexes with proteins and others components,
2. **binding**: no additional buffers required for nucleic binding on the particles,
3. **washing**: single wash with special *Wash* buffer for *SileksMagNA-Direct ™* is recommended to remove impurities associated with adsorbed nucleic acids. Further washing is carried out using distilled water.
4. **elution**: special *Elution* buffer for *SileksMagNA-Direct ™*.

3. Circulating Nucleic Acid (cNA)

DNA and RNA are integral parts of cells. A small amount of nucleic acids is detected circulating in the blood. These DNA and RNA molecules are formed from destroyed and living cells, the contents of which are released into the bloodstream. The presence of such circulating DNA and RNA allows the use of non-invasive diagnostic methods to identify a number of clinical diseases and disorders. Detection of specific DNA and / or RNA gives a unique possibility for early diagnostics of various pathologies:

- detect cancer in the early stages,
- viral infections,
- non-invasive perinatal diagnostics,
- identification of some forms and course of diabetes,
- much more.

Free circulating nucleic acids in plasma and serum are either in free unbound form or bound in the complexes with proteins, histones, bound on cell surfaces or cell wall residues, encapsulated inside vesicles.

Plasma is more often used for the isolation and analysis of cNA than serum. So, the procedure of preparing plasma for the isolation of cNA is one of the most important stage, ensuring the effectiveness of the subsequent isolation.

4. Plasma Isolation Protocol

4.1 Plasma preparation

Proper plasma preparation is a very important procedure.

During transportation and storage of a blood sample, cells can be damaged. As a result, an additional pool of nucleic acids is formed in the sample that are not related to the initially circulating nucleic acids (cNA). The presence of nucleic acids from blood cells damaged during the transportation process reduces the reliability and sensitivity of detecting true cNA.

To prevent the damage of blood cells during storage and transportation, we recommend using tubes with special stabilizers for blood sampling. Such stabilizers can be K_3EDTA, formaldehyde and other reagents that increase cell stability.

The most reliable way to prepare high-quality plasma is to separate the plasma immediately after receiving a blood sample and then store it at a temperature no higher than + 4°C. For long-term storage is recommended to freeze the prepared plasma to -80°C.

We recommend using the following procedure to prepare the plasma:

1. Centrifuge the blood sample at room temperature at 600 x *g* , 20 minute.
   Gentle centrifugation is necessary to prevent blood cells damage.

2. Carefully transfer the plasma to another tube. During the selection of plasma, avoid trapping cells collected at the interface.

3. Centrifuge the collected plasma at room temperature at 6000 x *g* , 20 minute.
   Re-centrifuging allows you to remove the remaining in the plasma cells.

4. Collect the plasma into a new tube.
   Store plasma at a temperature no higher than + 4°C.

Recommended plasma storage time (during frozen plasma storage, avoid freezing / thawing):

+4 °C - up to 2 weeks
-20 °C - up to 1 month
-80 °C - few years

4.2 cNA isolation from 2 mL plasma

1. Add **2 mL** of plasma or serum to 5 mL tube.
2. Add **2.4 mL Lysis&Denaturation Buffer**.
   Mix thoroughly.
3. Incubate for **20 minutes** at room temperature.
4. Add **5 µL** well mixed **SileksMagNA-Direct™** particles.
   Mix thoroughly.
5. Incubate for **10 minutes** at room temperature.
   Mix once or twice during incubation
6. Place the tube in a **magnetic rack** to collect the particles.
   Discard the supernatant.
7. Place the tube in a **non-magnetic rack**.
   Add **200 µL Wash Buffer**.
   Mix thoroughly until a homogeneous suspension is obtained.
   Transfer the resulting suspension to a 1.5 ml tube for further isolation.
8. Place the tube in a **magnetic rack** to collect the particles.
   Discard the supernatant.
9. Place the tube in a **non-magnetic rack**.
   Add **500 µL** ddH_2O.
   Mix thoroughly until a homogeneous suspension is obtained.
10. Place the tube in a **magnetic rack** to collect the particles.
    Discard the supernatant.
11. Repeat the procedure described in steps 9, 10, 2 times.
12. After removing the supernatant, place the tube in a **non-magnetic rack**.
    Add **50 µL Elution Buffer**.
    Mix thoroughly until a homogeneous suspension is obtained.
13. Incubate at **+80°C** for **5 minutes**.
14. Place the tube in a **magnetic rack** to collect the particles.
    Transfer DNA/RNA containing eluate to a clean tube.
15. Add **1 µL Fixation Buffer** to the collected eluate.
    If 100 µl of Elution buffer was used for elution, then 2 µl of Fixation buffer should be added after elution.

    Store the resulting solution with the isolated nucleic acid at -20°C.
    If the target of the isolation is RNA, we recommend as soon as possible after isolation to carry out the reverse transcription reaction, since dissolved RNA may degrade during storage.

5. Urine Isolation Protocol

5.1 Urine preparation

Urine should be collected according to standard established orders.

For better preservation of nucleic acids in the urine, we recommend using special tubes with stabilizers in which DNA and RNA in the urine can be stabile for a long time (more than 2 years).

We recommend using the following procedure for preparing cell-free urine:

1. Centrifuge the urine sample at room temperature at 600 x *g* , 20 minute.
   Gentle centrifugation is necessary to prevent cell damage and remove debris.

2. Carefully transfer the urine to another tube. During the selection of urine, avoid trapping precipitated cells.

3. Centrifuge the collected urine at room temperature at 6000 x *g* , 20 minute.
   Re-centrifuging allows you to remove the remaining in the urine cells and debris.

4. Collect the urine into a new tube.
   Store urine at a temperature no higher than + 4°C.

After receiving the urine sample, we recommend storing it at a temperature of + 4°C for no more than a day. For long-term storage, it is recommended to freeze urine at -80°C.

Recommended urine storage time (during frozen urine storage, avoid freezing / thawing):

+4 °C - up to 2 weeks
-20 °C - up to 1 month
-80 °C - few years

5.2 Isolation from 10 mL urine

We recommend using a volume of 10 ml, as optimal for most methods of analysis. In the case of other initial volume from the recommended one, change proportionally the amount only of the added *Lysis & Denaturation* buffer. The adding amounts of other components remain the same.

1. Add **10 mL** of urine to 50 mL tube.
2. Add **12 mL Lysis&Denaturation Buffer**.
   Mix thoroughly.
3. Incubate for **20 minutes** at room temperature.
4. Add **5 µL** well mixed **SileksMagNA-Direct™** particles.
   Mix thoroughly.
5. Incubate for **10 minutes** at room temperature.
   Mix periodicaly during incubation
6. Place the tube in a **magnetic rack** to collect the particles.
   Discard the supernatant.
7. Place the tube in a **non-magnetic rack**.
   Add **200 µL Wash Buffer**.
   Mix thoroughly until a homogeneous suspension is obtained.
   Transfer the resulting suspension to a 1.5 ml tube for further isolation.
8. Place the tube in a **magnetic rack** to collect the particles.
   Discard the supernatant.
9. Place the tube in a **non-magnetic rack**.
   Add **500 µL** ddH_2O.
   Mix thoroughly until a homogeneous suspension is obtained.
10. Place the tube in a **magnetic rack** to collect the particles.
    Discard the supernatant.
11. Repeat the procedure described in steps 9, 10, 2 times.
12. After removing the supernatant, place the tube in a **non-magnetic rack**.
    Add **50 µL Elution Buffer**.
    Mix thoroughly until a homogeneous suspension is obtained.
13. Incubate at **+80°C** for **5 minutes**.
14. Place the tube in a **magnetic rack** to collect the particles.
    Transfer DNA/RNA containing eluate to a clean tube.
15. Add **1 µL Fixation Buffer** to the collected eluate..
    If 100 µl of Elution buffer was used for elution, then 2 µl of Fixation buffer should be added after elution.

    Store the resulting solution with the isolated nucleic acid at -20°C.
    If the target of the isolation is RNA, we recommend as soon as possible after isolation to carry out the reverse transcription reaction, since dissolved RNA may degrade during storage.

6. Recommendations for Protocol Modification

1. We recommend to use *SileksMagNA-Direct™* particles in the following amounts:

100 µL to 2 mL starting plasma or serum sample	- 5 µL,
2 mL to 10 mL starting plasma or serum sample	- 10 µL,
1 mL to 15 mL starting urine sample	- 5 µL,
15 mL to 30 mL starting urine sample	- 10 µL.

However, depending on the quality of your sample and the tasks, it may be necessary to optimize the amount of magnetic particles.

2. For higher concentration of nucleic acid in the eluate is allowed to use a smaller volume of elution *Elution* buffer. The minimum allowable volume of *Elution* buffer must be at least 2 volumes to the volume of particles.
Fixation buffer should be added to the resulting eluate in the following amounts:
up to 50 µL of eluate - 1 µL *Fixation* buffer,
100 µL of eluate - 2 µL *Fixation* buffer.

3. For estimation of quality and quantity of isolated nucleic acids we recommend to give preference to methods based on PCR over methods for determining the amount of isolated nucleic acids on a spectrophotometer and other methods of optical detection. Use the proper normalization, especially when comparing the concentration of nucleic acids isolated with this kit and kits of other manufacturers. The influence of impurities and the presence of incorporates coprecipitants used by many others companies can make a false impression of the effectiveness of the isolation.

4. A distinctive feature of SileksMagNA-Direct™ magnetic particles is a low amount of inhibiting components. The resulting eluate may be completely (without dilution) used in the first strand synthesis reaction. The reaction components can be added directly to the eluate without the need to dilute the reaction mixture with water.

IMPORTANT NOTE

High-quality suspension of particles is the key to getting a good result.

To obtain high yield and purity of DNA / RNA, it is necessary to carefully resuspend particles at each stage of washing.

7. Comments

General notes

1 ml of plasma of a healthy person contains on average from 1 to 50 ng of circulating nucleic acid (cNA). The number of cNA increases in the plasma of sick people, especially in cancer patients, and in pregnant women..
The low concentration of isolated cNA makes senseless the use of spectrophotometric methods for the estimation of the resulted nucleic acid, even with the use of intercalating fluorescent dyes. The unique correct estimation method is quantitative PCR.
When designing primers and probes, it is necessary to take into account the strong fragmentation of cNA and the final amplicon size must not exceed 100 bp.
In the study of RNA, we do not recommend using oligo (dT) primers for the first strand synthesis. The use of randomized primers usually is increasing the sensitivity of the method. Some researchers prefer the use of gene-specific primers to increase the detection specificity, but this may reduce the sensitivity of the method.

Comments to the protocol items

1. Before using plasma or serum, make sure that it does not contain blood cells. If there are suspicions that blood cells are present in the plasma, repeat the plasma preparation procedure. (see *Plasma preparation*).
   When using frozen plasma (urine) after thawing the plasma (urine), mix it thoroughly.

2. Magnetic particles must be thoroughly mixed before use.

3. Particles for maximum sorption must be evenly distributed throughout the volume. During the incubation, make sure that the particles do not precipitate. If this occurs, mix the contents of the tube until a homogeneous suspension.

4. Long-term storage of isolated nucleic acid (DNA and RNA) bound on the particles surface allows to accumulate samples making a stop at elution stage followed by a simultaneous final isolation. This approach eliminates the possibility of damage to the isolated nucleic acid during storage.

5. To the first strand synthesis reaction can be taken any (up to maximal) amount of resulted eluate. A big amount of eluate will not inhibit the reaction.

   The storage of cNA, which is already largely fragmented, leads to even more fragmentation, up to complete destruction. Therefore, we strongly recommend to use nucleic acid for further work immediately after its isolation.

6. The binding of nucleic acids on the surface of the particles is very strong. Desorption is carried out in hard conditions: at elevated temperatures and using special reagents. In this case, the double-stranded form becomes single-stranded. As a result, the obtained nucleic acid cannot be used for the enzymatic cleavage of two-strand forms (restriction analysis). On electrophoresis, the result of the isolation will look like a smear or not visualized at all, and the results of the analysis using intercalating dyes also give an incorrect estimate of the concentration. Only estimation methods may be used: PCR, sequencing, hybridization techniques.

8. Related Products

1. **Lysis&Denaturation** Buffer, 250 mL, cat. No BLD250

2. Handheld mixer **LabMix Mini 201**, cat. No EQMM201

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

   MagRack6 Magnetic Rack, cat. No EQRM06
   MagRack16 Magnetic Rack, cat. No EQRM16
   MagRack40 Magnetic Rack, cat. No EQRM40

9. Contacts

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