SileksMagNA-Direct™ micro RNA Isolation kit, phenol-free (isolation from plasma, serum, saliva, spinal fluid, urine)

Cat. No: KIMIRNA0300

Application: micro RNA isolation from plasma, serum, saliva, spinal fluid, urine
Stirage: +4°C
Transportation conditions: at ambient temperature, special conditions are not required

Kit Content

Kit contains all essential components for 100 isolation procedures. The starting volume of sample – 300 µL.

Components

• **DEMIX** buffer	50 mL
• **APS** buffer	30 mL
• **SileksMagNA-Direct™** magnetic particles	1 mL
• **Elution** buffer	10 mL
• **Fixation** buffer	0.3 mL
• **RNA Protector**	0.5 mL

We are constantly working to improve the quality of our kits and regularly make changes in description that improve the functional quality of our kits. Please follow the current version of the description for the kit. Ask us about new changes to always get the best results.

Content:

1. Description

The kit is designed on base of SileksMagNA-Direct magnetic particles. SileksMagNA-Direct particles have a unique ability to capture extreme short oligonucleotides in length from 2-3 nucleotide bases. For the maximum efficiency to capture of fragments those are less than 10 nucleotides, we have developed a buffer composition that enture us an effective capture of such short oligos.

It is important that the kit isolates all nucleic acids from the sample - micro RNAs, RNAs, DNAs. The content of long nucleic acids can be lower compared to standard kits for RNA and/or DNA isolation. Despite the fact that are isolated all types of nucleic acids, the kit is optimized at first to isolate micro RNA. If it is necessary to remove DNA from the resulting material, we recommend using the DNAZA, as described in the protocol below.

In the isolation procedure, aggressive and toxic substances such as phenol, chloroform, and guanidine salts are not used. The washing procedure is very simple - captured on the magnetic particles nucleic acids mast be washed only with distilled water without loss of sorbed material. Sorbed nucleic acids can be stored, if necessary, in an aqueous suspension (from +4 ° C to room temperature) for a long time until the final isolation stage.

2. Important Information

- The isolation procedure can be scaled without loss of quality. It is important to keep a ratio of the following components used in the procedure: **Sample : DEMIX buffer : APS buffer**. The capture capacity of particles is excessive in relation to the amount of nucleic acids that are contained in sample, so the amount of added particles may be keeped the same as recommended in protocol.
- Working with a sample and reagents for safety purposes, use a medical mask that closes your mouth and nose, gloves and protective glasses.
- The nucleic acids received during the isolation process contains nucleic acid in a denatured single-chain form and it is not recommended for storage even after adding reagent RNA Protector. We strongly recommend using the received material on the same day. If necessary, store the received material no longer than 1-2 days at -20 °C or below.

3. Additional equipment and reagents that are not included in the kit

- Test tubes
- Vortex
- Centrifuge, speed of 12000 – 14000 rpm
- Magnetic rack for 1.5 mL tubes
- Water, Typ I, 18 MΩ /cm
- Thermoblock with a temperature +80 °C

4. Sample Preparation

Proper preparation of the sample plays an important role for the correct analysis of both the isolated micro RNA contained in extracellular vesicles and also for other extracellular nucleic acids. The main task in preparing the sample is to avoid the ingress of nucleic acids from cells that can be damaged in the process of collecting and transporting the sample.

Plasma Preparation

In the process of transportation and storage of a blood sample, cells can be destroyed. As a result, an additional pool of nucleic acids is formed in the sample, not related to the initially circulating nucleic acids (CNCs). The presence of nucleic acids from blood cells destroyed during transportation of blood cells reduces the reliability and sensitivity of the detection of true CNCs.

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

The best way to prepare high -quality plasma is the plasma separation, if possible, immediately after obtaining a blood sample with subsequent storage at a temperature of not higher than +4 ° C. For long -term storage, prepared plasma is recommended to freeze at -80 ° C.

We recommend using the following plasma separation procedure:

1. Centrifugate a blood sample at room temperature at 800 cpm, 20 minutes. Soft centrifugation is necessary to prevent damage to blood cells.

2. Carefully collect the plasma into another tube. When collecting plasma, avoid capturing cells on the interphase.

3. Centrifuge collected plasma at room temperature at 6000 cpm, 20 minutes. Repeated centrifugation allows to remove the remaining cells in plasma.

4. Collect the plasma into another test tube. Store plasma at a temperature not higher than +4 °C.

Recommended storage time for collected plasma (in the process of storing frozen plasma avoid freezing/thawing):

+4 °C - up to 1 month
-20 °C - up to 1 year
-80 °C - several years

Urine Preparation

Urine should be collected in accordance with standard established procedures.
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 stably stored for a long time (more than 2 years).

We recommend the following procedure to prepare urine free from cells:

1. Centrifugate a sample of urine at room temperature at 800 cpm, 20 minutes. Soft centrifugation is necessary to prevent cell damage and eliminate a debris.

2. Carefully collect the urine into another tube. When collecting urine, avoid capturing the pellet.

3. Centrifugate the collected urine at room temperature at 6000 cpm, 20 minutes. Repeated centrifugation allows you to remove the remaining cells and debris.

After receiving a urine sample, we recommend store it at a temperature of +4 °C no longer then 5 days. For long term storage of urine is recommended a storage at -80 °C.

Recommended time for urine storage (in the storage process of frozen urine avoid freezing/thawing):

+4 °C - up to 5 days
-20 °C - up to 1 month
-80 °C - several years

Saliva Preparation

Saliva should be collected in accordance with standard established procedures.
Sterile cotton swabs are used standardly to absorb saliva. The swab is then placed in a container with a transportation buffer and transported for further laboratory analysis.

We recommend to use the following procedure to prepare saliva free from cells:

1. Centrifugate a sample of saliva at room temperature at 8000 cpm, 20 minutes. Soft centrifugation is necessary to prevent cell damage and eliminate a debris.

2. Carefully collect the saliva into another tube. When collecting saliva, avoid capturing the pellet.

After receiving a saliva sample, we recommend store it at a temperature of +4 °C no longer then 5 days. For long term storage of saliva is recommended a storage at -80 °C.

Recommended time for saliva storage (in the storage process of frozen saliva avoid freezing/thawing):

+4 °C - up to 5 days
-20 °C - up to 1 month
-80 °C - several years

5. micro RNA Isolation

Isolation Procedure

The protocol describes the isolation procedure from plasma sample. The isolation from serum, saliva, urine, spin fluid is similar.

micro RNA isolation from plasma

1. Add **300 µL** of plasma in 1.5 ml tube.
2. Add **450 µL** of **Demix** buffer.
3. Mix the solution thoroughly by pipetting.
4. Incubate at room temperature during **5 minutes**.
5. Add **450 µL** of **APS** buffer.
6. Close the tube and thoroughly mix the resulting suspension on the vortex.
7. Incubate at room temperature during **5 minutes**.
8. Centrifugate at **12'000 cpm** (or 14'000 cpm) during **5 minutes**.
9. Transfer the supernatant into another tube. Be careful not to capture the pellet.
10. Add **5 µL** of well -mixed particles **SileksMagNA-Direct™**.
    Mix thoroughly by pippeting.
11. Incubate **5 minutes** at room temperature periodic pipetting.
12. Place the tube in a **magnetic rack** to collect particles (it may take up to 1 minute).
13. Remove supernatant. Be careful not to capture magnetic particles.
14. Place the tube in a **non-magnetic rack**.
15. Add **1000 µL** of distilled water. Mix this mixture thoroughly.
16. Place the tube in a **magnetic rack** to collect particles (it may take up to 1 minute).
17. Remove supernatant. Be careful not to capture magnetic particles.
18. Place the tube in a **non-magnetic rack**.
19. Add **200 µL** of distilled water.
20. Mix thoroughly until a homogeneous suspension is obtained.
21. Place the tube in a **magnetic rack** to collect particles. Remove supernatant.
22. After removing the supernatant, place the tube in a **non-magnetic rack**.
23. Add **30 µL** of **Elution** buffer.
24. Mix thoroughly until a homogeneous suspension is obtained.
25. Incubate the tube in thermoblock at **80°C** during **5 minutes**.
26. Place the tube in a **magnetic rack** to collect particles.
27. Transfer the eluate containing micro RNA to a clean tube.
28. Add **2 µL** of **Fixation** buffer to the collected eluate.

To slowing a degradation of the received nucleic acids, add **3 µl** of **RNA Protector** to the eluate.

Keep in mind, obtained nucleic acids by storage at -20 ° C can degradate.

We recommend use the obtained material on the same day.

If it is necessary to remove the interfering DNA from the resulting material, we recommend to use the protocol below.

Protocol of removing of DNA using DNase I (calculated on the final volume of 100 µl)

1. Prepare the mixture, consisting of:
   - 10 µL of sample
   - 10 µL of 10X buffer for DNase I
   - 1 µL of DNase I (appr. 2 u)
   - 79 µL of deionized sterile water
2. Incubate 10 minutes at +37 °C.
3. Inactivate enzyme during 10 minutes at +75 °C.

To remove DNase, we also recommend use our kit "Cleaning of enzymatic reactions products", cat. number K0900 (purchased separately).

6. Comments

General comments

The proposed kit is optimized for the isolation of micro RNA. The preservation of a proportional amount of other accompanying nucleic acids during isolation is not the task of this kit and can vary from the type of sample.

Comments to the section Sample Preparation

The methods of preparing samples given in the section are only recommended, but not mandatory.
Other methods for preparing samples or other conditions (other buffers, centrifugation parameters, etc.) can be used. The basic requirements for a prepared sample - in the prepared sample should not contain impurities of nucleic acids of cells destroyed during the manipulation with sample.

Comments to the section micro RNA Isolation

The protocol is developed to isolation from 300 µL of sample.
If a sample of smaller volumes is used, we recommend to scale the isolation in accordance with the table below.

Table 1. Scaling the protocol depending on the initial volume of sample

Starting Sample, µL	DEMIX buffer, µL	APS buffer, µL	Mag. particles, µL	Wash 1, H_2O, µL	Wash 2, H_2O, µL	Elution buffer, µL	Fixation buffer, µL	RNA Protector, µL
100	150	100	5	400	200	30	2	3
200	300	200	5	800	200	30	2	3
300	450	300	5	1000	200	30	2	3

Operating with plasma sample, after adding APS buffer, is observed the most strong formation of a suspension from all types of samples (saliva, urine). After centrifugation and precipitation of the resulting suspension, the supernatant should be transparent and have color from colorless to light yellow.

To eluate micro RNA collected on SileksMagNA-Direct particles must be used only special Elution buffer for these particles. It is necessary add Fixation buffer to an eluate after elution.

To desorb micro RNA with water or with TE buffer is impossible!

If it is necessary to remove DNA, we recommend, after the isolation, additional treatment of the sample by the DNase enzyme, followed by the mandatory reprecipitation of the remaining nucleic acids. To remove DNase, we also recommend use our kit "Cleaning of enzymatic reactions products", cat. number K0900 (purchased separately).

In further work with micro RNA, we recommend the following rules:
add the resulting eluate in the reaction of synthesis of the first chain in an volume of not more than 1/8 of the volume from the final volume of the reaction mixture.
The recommended volume added into the reaction is 1/10 of the volume.
For example, when the reaction of synthesis of the first chain is prepared in a volume of 25 µl, the recommended volume of eluate is 2.5 µl. The use of more eluate can lead to inhibiting of the reaction of synthesis of the first chain and, as a result, a decrease in the sensitivity of micro RNA detection in subsequent the polymerase chain reaction.

Appendix 1

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 or heating, as in case of standard RNase inhibitors of protein nature.

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 eluate.

For example, if 30 µL of Elution buffer was used, after elution and collection of the sample it is necessary to add 3 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).

6. Related Products

- LabMix Mini 201 handheld mixer, cat. No: EQMM201
  allows to achieve as thorough mixing as it is with manual pipetting. It also increases results reproducibility.

- Magnetic racks for operating with magnetic particles:
  MagRack6, cat. No: EQMR006
  MagRack16, cat. No: EQMR016

7. Contacts

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