SileksMagNA™ FFPE DNA/RNA Isolation Kit

Catalog No KRFF100
100 DNA/RNA isolation reactions from formalin-fixed, paraffin-embedded samples
Research use only

Shipping: 0 to +10 ºC
Storage: -20 ºC for Proteinase
+4 ºC for all other kit components
Can be stored at least for one year

Kit Contents

Components (all are ready-to-use)	Volume
Proteinase	1 ml
Proteinase Buffer (1x)	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

Related Equipment and Reagents by Sileks

Cat. No MPR001 SileksMagNA™ Magnetic Particles
Cat. No EQRM06 MagRack6 Magnetic Rack
Cat. No EQRM16 MagRack16 Magnetic Rack
Cat. No EQRM40 MagRack40 Magnetic Rack
Cat. No EQMM201 LabMix Mini 201 Mixer

Safety (more information in product material safety data sheet (MSDS))

- When working with chemicals, wear a lab coat, disposable gloves, and protective goggles. Some components may cause health damage if swallowed, breathed, in contact with eyes or skin. Follow MSDS instructions for safety.
- Avoid mixing the kit components with strong acids and alkali.
- In case of contact with eyes, rinse immediately with plenty amount of water. In case of contact with skin, immediately wash the skin with soap and plenty amount of water.

SileksMagNA™ FFPE DNA/RNA Isolation Kit

Advantages

- High DNA/RNA yield and consistent results
- High purity (>1.8 OD_{260}/OD_{280}) DNA/RNA, excellent for molecular biology applications such as PCR, reverse transcription, sequencing, restriction digestion, sequencing on chips, hybridization on chips etc.

Applications

- Rapid and efficient isolation of various DNA/RNA from formalin-fixed, paraffin-embed tissue samples
- Separate isolation of DNA and RNA as well as simultaneous isolation of DNA/RNA

Principle

SileksMagNA™ FFPE DNA/RNA Isolation Kit is designed for efficient and rapid (120 min) isolation of DNA/RNA from formalin-fixed, paraffin-embedded (FFPE) samples using SileksMagNA™ silica-covered magnetic particles. All components of the kit are ready to use and there is no need to supply additional reagents for preparing wash buffers. For deparaffinization stage xylene (not included in the kit) must be used. Provided components are sufficient to perform 100 simultaneous DNA/RNA isolation procedures or for 50 isolations of RNA and DNA in separate tubes.
Isolation procedure with SileksMagNA™ FFPE DNA/RNA Isolation Kit consists of a few short and simple steps:

- Sample deparaffinisation
- Proteinase digestion
- Sample lysis: remaining proteins are denaturated and unbound from nucleic acids
- Binding: nucleic acids bind to magnetic particles
- Washing: residual contaminants are washed away while pure nucleic acids remain bound to magnetic particles
- Drying: incubation at 60°C removes traces of washing buffers
- Elution: purified nucleic acids are eluted from magnetic particles

SileksMagNA™ FFPE DNA/RNA Protocol for simultaneous or separate isolation of DNA and RNA

Important Notes

- SileksMagNA™ magnetic particles are particles for isolation of total nucleic acids (both DNA and RNA). To obtain only RNA after purification, use conventional DNase treatment to remove DNA. If you intend to use DNA after the purification, use conventional RNase treatment to remove RNA.

Follow the complete procedure for simultaneous DNA/RNA isolation. But if you want to isolate RNA and DNA in separate tubes, divide the sample after deparaffinisation into two tubes and treat two samples separately as described below.

- DNA/RNA isolated from FFPE samples is typically much more fragmented compared to other samples such as blood or fresh tissue. Take it into account when designing amplification systems. Typical DNA fragments are in a range of 200 bp, RNA fragments are in a range of 100 b.
- Precipitate may form in some buffers; however, this has no effect on the quality. Warm up the buffer at 50°C to dissolve precipitate if formed. All buffers must be mixed well before use to produce a uniform suspension.
- "*Mix thoroughly*" in the protocol means that the solution must be mixed either by manual pipetting (20 times) or using LabMix mixer for 5 seconds on low/medium speed. "*Mix well*" means that the solution must be shaken 5-10 times.

Protocol

Deparaffinization

1. Add **1 ml** of **xylene** to the tube, containing a FFPE section. Vortex for 10 seconds. Incubate at room temperature for 10 minutes. Vortex the tube during the incubation at least 2 times. Centrifuge the tube at 12,000-18,000 × g in a microcentrifuge 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 as described in step 1.
3. Add **1 ml** of **96% ethanol**. Vortex for 10 seconds. Centrifuge the tube at 12,000-18,000×g in a microcentrifuge for 2 minutes. Carefully remove the supernatant.
4. Repeat step 3 once more.
5. Add **1 ml** of **70% ethanol** and repeat vortexing, centrifugation and supernatant removal as described in step 3.
6. Keep the tube lid open for 10 minutes to dry the pellet.

Proteinase digestion (pretreatment)

For simultaneous DNA and RNA isolation proceed by the following: first carry-out Pretreatment for RNA isolation, collect the supernatant and use it for further Purification on magnetic particles step to isolate RNA. Then proceed with the pellet to Pretreatment for DNA isolation and follow further Purification on magnetic particles step to isolate DNA.

For simultaneous DNA and RNA isolation you will require a double amount of the reagents compared to single RNA or DNA isolation.

For RNA sample perform only Pretreatment for RNA isolation followed by Purification on magnetic particles.

For DNA sample perform only Pretreatment for DNA isolation followed by Purification on magnetic particles.

Pretreatment for RNA isolation (used for simultaneous DNA/RNA isolation or for separate RNA isolation):

7. Add **100 µl** of **Proteinase Buffer** and **10 µl** of **Proteinase** to the tube, containing a sample after deparaffinization. Vortex and incubate at **+60 ºC** for at least **1 hour**. Depending on the tissue material, the sample may be lysed not completely. It does not affect the result.
8. Incubate the tube at **+80°C** for **30 minutes**. Maximum incubation time can be up to 60 minutes. Longer incubation time leads to more severe fragmentation of RNA.
9. Centrifuge the tube at **12,000-18,000×g** for **10 minutes**.
10. Transfer RNA-containing supernatant to a fresh tube without disturbing the pellet. Follow **Purification on magnetic particles** step to isolate RNA (RNA or DNA and RNA isolation protocol) or proceed with the pellet to isolate DNA (DNA and RNA isolation protocol).

Pretreatment for DNA isolation (used for simultaneous DNA/RNA isolation or for separate DNA isolation):

11. Add **100 µl** of **Proteinase Buffer** and **10 µl** of **Proteinase** to the tube, containing the pellet from the step 10 (DNA and RNA isolation protocol) or a sample after deparaffinization (DNA isolation protocol). Vortex and incubate at **+60 ºC** for at least **1 hour**. Depending on the tissue material, the sample may be lysed not completely. It does not affect the result.
12. Heat up the tube to **+90 ºC**. Incubate for **2 hours**.
13. Centrifuge the tube at **12,000-18,000×g** for **10 minutes**.
14. Transfer DNA-containing supernatant to a fresh tube without disturbing the pellet. Follow **Purification on magnetic particles** step to isolate DNA.

Purification on magnetic particles

15. Add **120 µl** of well mixed **START Buffer** to 100 µl of supernatant obtained after pretreatment stage and mix thoroughly.
16. Incubate at room temperature for **5 minutes**. Incubation up to 15 minutes increases DNA/RNA yield.
17. In a separate tube mix the following components: **240 µl** of well mixed **Lysis & Binding Buffer** and **5 µl** of well mixed **SileksMagNA™ Magnetic Particles**. Mix thoroughly.
18. Add the prepared suspension of magnetic particles to the tube, containing prepared sample. Mix thoroughly. Incubate for **5 minutes** at room temperature, mix once or twice during incubation.
19. To collect magnetic particles with bound DNA/RNA, place the tube in a **magnetic rack** for **1 minute**. Discard the supernatant. Be careful not to disturb magnetic particles collected at the tube wall.

20. 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.
21. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
22. 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.
23. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
24. Place the tube in a **non-magnetic rack**. Add **300 µl** of **Wash 3 Buffer** and mix thoroughly until a homogeneous suspension is obtained.
25. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
26. Place the tube in a **non-magnetic rack**. Add **300 µl** of **Final Wash Buffer** and mix thoroughly.

You can stop the procedure at this stage for best DNA and RNA preservation. Long-term storage of DNA and RNA bound to magnetic particles in Final Wash Buffer is the safest option, excellent for DNA and RNA conservation or transportation purposes. To use isolated DNA and RNA after years of storage at -20ºC the procedures listed below must be carried out to obtain pure DNA and RNA.

27. Place the tube in a **magnetic rack** to collect the particles. Discard the supernatant.
28. Incubate the tube with its lid open in a thermal block at **60°C** for **5 minutes** to dry the pellet of magnetic particles.
29. Add **50 µl** of **Elution Buffer**. Thoroughly resuspend particles until a homogeneous suspension is obtained. If you wish to have higher DNA/RNA concentration, use 25 µl of Elution Buffer.
30. Incubate in a thermal block at **60°C** for **5 minutes**.
31. Place the tube in a **magnetic rack** to collect the particles. Transfer the supernatant containing DNA/RNA to a fresh tube. To get rid of all particles, repeat the same with the eluate: place in a **magnetic rack** and transfer supernatant into a new tube.
32. For simultaneous DNA/RNA isolation and for separate RNA isolation cases, add $^{1}/_{10}$ volume (5 µl) of **RNA Protector** solution to preserve RNA. Skip this stage if you are only purifying DNA.
33. The isolated DNA/RNA mixture can be stored at -20°C or used directly.
34. DNA is stable and suitable for a long storage. If required, treat it with RNase to remove RNA and use it for subsequent applications.
35. However, if your intention is to work with RNA, avoid long storage, as RNA is prone to degradation. Use RNA for cDNA synthesis as soon as you can. Remove DNA by DNase treatment, if necessary.
36. Check the quality of DNA and RNA on appropriate agarose gel if needed.

Troubleshooting

Problem	Possible causes	Solution
Low DNA/RNA yield	The sample contains too small amount of DNA/RNA or the sample is old.	Take 2x bigger sample volume for isolation. Try to perform elution in smaller volume of *Elution* buffer. If it doesn't help, DNA/RNA in the sample are irreversibly degraded, conditions of formalin fixation and paraffinization must be optimized.
	Too much starting material	Use recommended amounts of samples and all buffers. Too much initial material reduces DNA/RNA yield due to suboptimal lysis, binding and wash.
	Incomplete drying of particles before adding Elution Buffer.	Increase the drying time in thermal block after removing Final Wash buffer. Check if all liquids are evaporated before elution.
	Incomplete lysis	Mix the sample more thoroughly after adding START Buffer.
	Too big volume of Elution Buffer used	Adjust optimal amount of Elution Buffer to provide required DNA/RNA concentration.
OD_{260}/OD_{280} ratio is too low	Protein contamination	Mix all buffers and the sample itself during the procedure as recommended in each step of the protocol.