SileksMagNA™ Cell Culture DNA/RNA Isolation Kit

Catalog No KRCC100
100 DNA/RNA isolation reactions from cell cultures
Research use only

Shipping: ambient temperature
Storage: +4ºC at least for one year

Kit Contents

Components (all are ready-to-use)	Volume
SamplePrep Buffer	50 ml
START Buffer	12 ml
Lysis & Binding Buffer	16 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	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™ Cell Culture DNA/RNA Isolation Kit

Advantages

- High DNA/RNA yield and consistent results
- High purity (>1.7 OD_{260}/OD_{280}) DNA/RNA, excellent for molecular biology applications such as PCR, cDNA synthesis, labeling, cloning, hybridization on chips, sequencing etc.
- Simultaneous isolation of RNA and DNA allows for easy normalization of results of gene expression analysis.

Applications

- Rapid and efficient isolation of DNA/RNA from cell cultures.
- DNA/RNA isolation from both suspension or adherent cell cultures.

Principle

SileksMagNA™ Cell Culture DNA/RNA Isolation Kit is designed for efficient and rapid (30 min) isolation of DNA/RNA from cell cultures 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. Provided components are sufficient to isolate high yield DNA/RNA from 100 standard samples. Single isolation provides up to 7 μg of pure DNA/RNA.
Isolation procedure with SileksMagNA™ Cell Culture DNA/RNA Isolation Kit consists of a few short and simple steps:

- Sample lysis: cells are destroyed and all cell components and nucleic acids are released into the solution
- 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™ Cell Culture DNA/RNA Isolation Protocol

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.
- The kit is optimized for isolation from approx. 10^5 cells. Optimization may be required in each individual case. A general advice is to use as small quantity of cells as needed to provide reliable and repeatable detection of the results. The number is usually in range from 0.5×10^5 to 5×10^5 cells per sample.
- 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

1. Transfer **0.5×10^{5}-5×10^5** cells as suspension in media to a fresh 1.5 ml tube. For adherent cell culture, use one of standard ways for detaching cells (for example trypsin-EDTA treatment).
2. Centrifuge the tube at **3,000-5,000** × g for **3 minutes**. Carefully remove the supernatant. Do not disturb the pellet of cells.
3. Add **500 µl** of **Sample Prep Buffer**. Resuspend cells in the buffer to wash them from media.
4. Centrifuge the tube at **3,000-5,000** × g for **3 minutes**. Carefully remove the supernatant. Do not disturb the pellet of cells
5. Add **120 µl** of well mixed **START Buffer** and mix thoroughly
6. Incubate at **room temperature** for **5 minutes**. Incubation up to 15 minutes increases DNA/RNA yield.
7. Centrifuge the tube at **12,000-18,000** × g for **3 minutes**.
8. In a separate tube mix the following components: **160 µl** of well mixed **Lysis & Binding Buffer** and **7 µl** of well mixed **SileksMagNA™ Magnetic Particles**. Mix thoroughly.
9. To the tube with the prepared suspension of magnetic particles in **Lysis & Binding Buffer** add **100 µl** of clear cell supernatant after centrifugation. Mix thoroughly. Leave at room temperature for **5 minutes** for binding.
10. 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.
11. Transfer the tube to a nonmagnetic rack. Add **300 µl** of well mixed **Wash 1 Buffer**. Mix thoroughly.
12. Put the tube in a magnetic rack. Wait for particles to collect completely. Discard the supernatant.
13. Repeat steps 11-12 using **300 µl** of well mixed **Wash 2 Buffer** instead of Wash 1 buffer.
14. Repeat steps 11-12 using **300 µl** of well mixed **Wash 3 Buffer** instead of Wash 1 buffer.
15. Transfer the tube to a nonmagnetic rack. Add **300 µl** of **Final Wash Buffer**. 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.

16. Put the tube in a magnetic rack. Wait for particles to collect completely. Discard the supernatant.
17. Put the tube (with its lid open) in a thermal block and dry the pellet at **60°C** for **10 minutes**.
18. Add **50 µl** of **Elution buffer**. Resuspend the pellet of particles. If you need to obtain higher DNA/RNA concentration, decrease Elution buffer volume to 25 µl.
19. Close the tube and put it in a thermal block. Incubate at **60°C** for **10-15 minutes**.
20. Resuspend the pellet of particles. Put the tube in a magnetic rack. Wait for particles to collect completely. Collect the supernatant, containing DNA/RNA, and transfer it to a fresh tube. Avoid collecting all the liquid because some portion of particles can be captured with the last microliters.
21. The isolated DNA/RNA mixture can be stored at -20°C or used directly. DNA is stable and suitable for a long storage. If required, treat it with RNase to remove RNA and use it for subsequent applications. 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. Check the quality of DNA and RNA on appropriate agarose gel if needed.

Troubleshooting

Problem	Possible causes	Solution
Low DNA/RNA yield	Not enough DNA/RNA-containing cells were collected.	Use bigger amounts of source material or elute DNA/RNA in a smaller volume of *Elution* buffer.
	Particles were not dried thoroughly enough before the elution.	Increase the drying time in thermal block after the *Final Wash* step.
	Lysis process not completed.	Mix the sample more thorough after adding *START* buffer.
	Too big volume of *Elution* buffer added.	Find optimal buffer volume to achieve the required concentration of DNA/RNA.
OD_{260}/OD_{280} ratio is too low	High protein contamination	Try to achieve better particles resuspension on each step of the isolation procedure. Use smaller amount of particles or bigger volume of *Elution* buffer.