

ClassiX CleanUp

(REF: CXCU-10mL)

Instructions for Use

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Intended use

ClassiX™ CleanUp is intended to be used for size-selective purification of nucleic acids in molecular biology research applications. Not for diagnostic use.

Principles of the method

ClassiX CleanUp is a suspension of paramagnetic particles, which is mixed with a DNA/RNA-containing sample for purification of nucleic acids. The protocol is based on the principle of solid-phase reversible immobilization (SPRI). The principle behind SPRI is a binding nucleic acids to paramagnetic carboxy-coated microparticles in the presence of a sufficient concentration of polyethylene glycol (PEG) and sodium chloride. The presence of PEG and NaCl cause DNA fragments to precipitate and attach to the microparticle's surface. Thereby, the size range of the nucleic acids bound to the particle surface is determined by the concentration of PEG as well as NaCl.

The ClassiX CleanUp microparticle suspension is ready-to-use because it contains already the required PEG and NaCl for nucleic acid binding. The desired size range of DNA/RNA to be purified can be adjusted by selecting the appropriate mixing ratio between ClassiX CleanUp suspension and sample to be purified. Through application of a magnetic field, the paramagnetic particles can be separated from the liquid phase, allowing for the removal of impurities while the desired DNA fragments can later be recovered by elution with a buffer of choice.

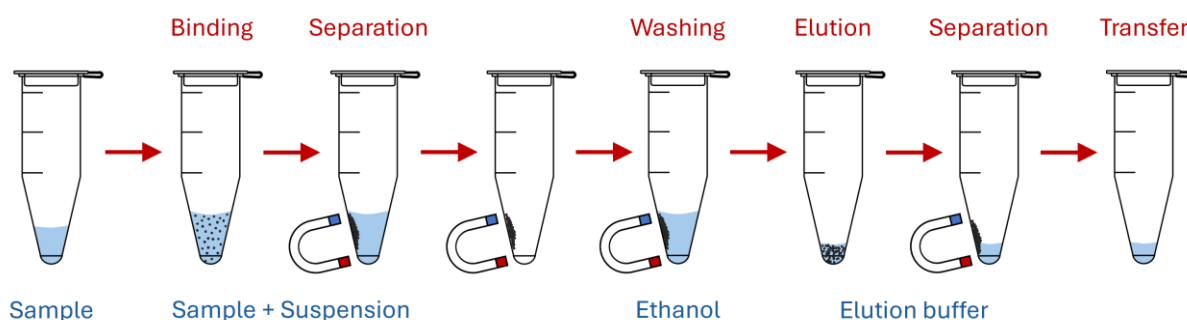
Equipment to be provided by the user

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- 2 mL or 1.5 mL microcentrifuge tubes
- Magnetic stand (Xpedite Diagnostics REF: MAG-12)
- Vortexer (Xpedite Diagnostics REF: VOR-01)
- Mini spin centrifuge (Xpedite Diagnostics REF: CEN-01)
- 70 % to 80 % (v/v) ethanol (prepare solution shortly before use for optimal results)
- Elution buffer: Molecular grade water, Tris-acetate buffer (10 mM pH 8.0) or TE buffer (10 mM Tris-acetate pH 8.0, 1 mM EDTA) can be used.

Storage and shelf life

ClassiX CleanUp must be stored at 2 °C to 8 °C. Do not freeze. Reagents are good to be used until the expiry date indicated on the label. Do not use reagents after their indicated expiry date. After first use, ClassiX CleanUp is good to be used within 3 months.

Graphical representation of the procedure



Determination of a suitable ratio between suspension and sample

Ratio of Classix CleanUp to sample	Example to illustrate the volume ratio	Fragment lengths binding to the microparticles
0.5x	Add 10 μL ClassiX CleanUp to 20 μL sample	≥ 1000 bp
0.8x	Add 16 μL ClassiX CleanUp to 20 μL sample	≥ 400 bp
1.0x	Add 20 μL ClassiX CleanUp to 20 μL sample	≥ 250 bp
1.2x	Add 24 μL ClassiX CleanUp to 20 μL sample	≥ 200 bp
1.8x	Add 36 μL ClassiX CleanUp to 20 μL sample	≥ 100 bp

Advice on sample input type and volumes

Generally, ClassiX CleanUp can be applied to purify virtually any sample input volume. You need to adjust the volume of ClassiX CleanUp suspension to (i) the sample input volume and (ii) the reagent ratio required for the desired size selection. We have validated sample input volumes ranging from 10 μL to 50 μL .

Apart from cleaning up PCR amplicons and NGS libraries, ClassiX CleanUp can also be used to purify and concentrate DNA/RNA lysates from rapid extraction kits such as SwiftX™ Virus. For that, either the whole lysis volume, e.g. 100 μL , can be cleaned or just a fraction of it. No matter what your choice is, always select the right ratio between sample volume and ClassiX CleanUp suspension using the above table.

Advice on elution buffer types and volumes

The purified nucleic acids can be eluted from the paramagnetic microparticles using various low-salt buffers, such as Tris-acetate (10 mM pH 8.0) or Tris-acetate/EDTA (10 mM Tris-acetate pH 8.0, 1 mM EDTA). If your downstream analytical method is very sensitive to the ionic strength of the eluate, then molecular-grade water may be the better choice for the nucleic acid elution.

We have validated sample input volumes ranging from 10 μL to 50 μL .

Step-by-step protocol for nucleic acid purification

Important things to do before starting:

- Let ClassiX CleanUp suspension warm up to reach room temperature.
- Shake or vortex ClassiX CleanUp for 30 seconds to homogenize the particle suspension.
- **Consult the above table** for determining the correct ratio of ClassiX CleanUp to be mixed with your sample to achieve the desired size-selective purification.








1. **Add the nucleic acid sample to be purified to a microtube.**
2. **Add the required volume of ClassiX CleanUp suspension and mix well by vortexing for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**
3. **Incubate microtube at room temperature for 5 minutes.**
4. **Place microtube in a magnetic stand for at least 2 minutes.**

Important: If the particles are not fully separated then extend the time before proceeding to the next step.
5. **Open microtube while remaining in the magnetic stand. Remove and discard the supernatant by pipetting.**

Expert tip: Leave about 5 μL of supernatant in the tube to avoid losing microparticles.
6. **Leave tube in the magnetic stand. Rinse the microparticles by carefully dispensing 200 μL of ethanol (70 % to 80 %) onto the pellet. Do not disturb the pellet!**

Important: If the total volume in Step 2 exceeds 200 μL then use the same volume of ethanol for the first wash step.
7. **Incubate microtube at room temperature for 30 seconds.**
8. **Remove and discard the supernatant by pipetting. Make sure to completely remove the liquid.**
9. **Repeat Steps 6 to 8.**
10. **Optional: Incubate the microtube in the magnetic stand for 1 minute with lid opened.**
11. **Remove microtube from the magnet stand and add a desired volume (10 μL to 50 μL) of elution buffer and mix well by vortexing of 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**
12. **Incubate microtube at room temperature for 2 minutes.**
13. **Place microtube in a magnetic stand for 2 minutes.**
14. **Transfer supernatant with purified size-selected nucleic acids to a new microtube.**

Key to symbols

	Catalog number
	Number of extractions
	Storage temperature
	Batch number
	Expiry date
	Read Instructions for Use
	Legal manufacturer

Legal manufacturer

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