A&A BIOTECHNOLOGY
innovating life science

Clean-Up

Kit for DNA cleanup after PCR and other enzymatic reactions using restriction enzymes, ligase, kinase, etc.

version 1118

50 isolations, 250 isolations

Cat. # 021-50, 021-250

The binding capacity of the purification minicolumn - up to 20 µg of DNA,
the minimum binding capacity - 2 µg of DNA (below 2 µg of DNA we recommend using of Clean-Up Concentrator Kit).
DNA fragments range - from 100 bp to 10 000 bp.
Typical DNA recovery - 60-90%.
Elution volume - 30-50 µl.

For R&D se only.

Kit Contents

Component	50 isolations	250 isolations	Store at
Minicolumns	50 pcs	250 pcs	Room Temp.
GI binding solution	45 ml	210 ml	Room Temp.
A1 wash solution	50 ml	250 ml	Room Temp.
Sodium acetate (3 M, pH 5.5)	1 ml	3 x 1 ml	Room Temp.
TE buffer	5 ml	16 ml	Room Temp.

It's not necessary to remove the mineral oil overlay if added to the enzymatic reaction mixture.

Equipment and materials necessary for DNA purification that are not included in the kit

1. DNA sample after enzymatic reactions
2. Sterile water (nuclease free, DEPC treated) (cat. # 003-075, 003-25, 003-500) (option)
3. Sterile 1.5 ml Eppendorf tubes
4. Centrifuge
5. Vortex (option)

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Purification protocol

1. Mix DNA samples (max 150 µl) with 5 volumes of GI binding solution. Mix by inverting the tubes or vortexing.

optimal condition pH ≤ 7.2

Binding solution GI contains the colour pH indicator.
Upon mixing the DNA sample with binding solution GI, yellow colour of the mixture indicates an optimal pH for DNA binding.

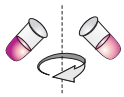
If the mixture colour turns pink, pH of the solution is too high.
In such conditions DNA binds inefficiently to the silica membranes and may be lost.

too high pH

If the colour of the solution is pink adjust the pH by adding 1–10 µl of 3M sodium acetate solution (pH 5.5) (included) and mix.
As soon as the colour of the mixture turns yellow, proceed with the purification protocol.

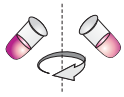
It is not necessary to remove the mineral oil overlay if added to enzymatic reaction mixture.

2. Briefly centrifuge the samples to remove the leftovers of solution from the tube walls and caps.



3. Apply the mixture onto the minicolumns.

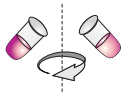
4. Centrifuge for 30 s at 10 000–15 000 RPM.



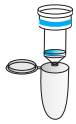
5. Remove the minicolumns from the tubes.
Discard the filtrates and re-attach the minicolumns to the same tubes.

6. Add 600 µl of A1 wash solution.

7. Centrifuge for 30 s at 10 000–15 000 RPM.

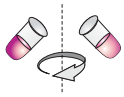


8. Remove the minicolumns from the tubes.
Discard the filtrates and re-attach the minicolumns to the same tubes.



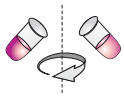
9. Add 300 µl of A1 wash solution.

10. Centrifuge for 1 min at 10 000–15 000 RPM.



11. Remove the minicolumns from the tubes.
Discard the filtrates and re-attach the minicolumns to the same tubes.

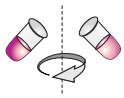
12. Centrifuge for 1 min at 10 000–15 000 RPM.



13. Transfer the dry minicolumns to the new 1.5 ml elution tubes (not included). Add 50 µl of TE buffer or sterile water (not included) directly onto the minicolumns resin.

Applying elution liquid (TE buffer or sterile water) onto the minicolumn be sure that liquid is applied directly onto the resin.
If some of liquid stays on the column walls the elution will be less effective.
Elution in a smaller volume is less efficient, but the extracted DNA has a higher concentration. Elution in 50 µl volume is more efficient, but DNA has a lower concentration.

14. Incubate for 3 min at room temp.
Centrifuge for 60 s at 10 000–15 000 RPM.



15. Remove the minicolumns.
The purified DNA store at +4 °C to +8 °C.

Safety information

DANGER
A1 wash solution
H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

WARNING
GI binding solution
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.