(Version 1.3)

DNA Clean - Purification after enzyme reactions

Cat No: MB19-22010 and MB19-22050

INTENDED USE and PROTOCOL OVERVIEW

NS Reagents DNA Clean is for the rapid purification of DNA after DNA reactions, removing enzymes and other contaminants in the process. Providing up to 99% recovery, it can be used for DNA fragments, plasmid DNA and genomic DNA from 50 bp to 20 kb, with optimum recovery for lengths of 100 bp to 10 kb. Primers are removed naturally as part of the process.

The DNA purification procedure uses spin columns which bind nucleic acids. The Bind Buffer breaks down proteins and goes pink if the pH is too high for optimal DNA binding (see detailed protocol). The wash steps remove impurities and enzyme inhibitors and the purified DNA is then eluted. Purified DNA can be eluted in TAE or TBE buffer or water and used in most downstream applications such as PCR, qPCR, sequencing etc or stored for later use.

COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS
Catalogue number	MB19-22010	MB19-22050
Bind Buffer	4 ml	20 ml
Wash Buffer	3.5 ml *Add 14ml of 96%-100% ethanol before use*	16 ml *Add 64ml of 96%-100% ethanol before use*
Elution Buffer (EDTA Free)	2 ml	10 ml
Columns	10 pcs	50 pcs
2ml Collection Tubes	10 pcs	50 pcs
Load Buffer (6X)	1 pc	1 pc

The kit can be stored at room temp (15-25°C) - Shelf life: At least 12 months when stored correctly.
All bottles should be closed tightly when not in use

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

96–100% ethanol PFA
1.5–2 ml microcentrifuge tubes
Vortex mixer
Microcentrifuge (≥ 11 000 x g)
3M sodium acetate, pH 5.2 (may be required)
Pipettes and tips (sterile filter tips are recommended)
Standard lab PPE equipment (lab coat & gloves)
Vortex mixer

SAFETY INFORMATION and PRECAUTIONS

Ensure protective clothing and equipment is used as appropriate.

Guanidine salt residues in the Bind Buffer may form highly reactive compounds when combined with oxidation components. In the event of a spillage, clean surfaces with a detergent water solution.

Binding Buffer: Warnings
H225, H302, H315, H319, H336
P210, P264, P280, P301+P312 P330

H225 Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H336** May cause drowsiness or dizziness. **P210** Keep away from heat/sparks/open flames/hot surfaces. No smoking. **P264** Wash hands thoroughly after handling. **P280** Wear protective gloves/protective clothing/eye protection/face protection.

P301+P312 P330: IF SWALLOWED: Call a POISON CENTER/DOCTOR if you feel unwell. Rinse mouth.

QUALITY CONTROL and KIT SPECIFICATIONS

The quality of each production batch of **NS Reagents DNA Clean** is tested with a range of DNA fragment lengths (100 bp - 10 kb) and masses . The Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

The Certificate of Analysis (CofA/COA) is provided in the form of the attached label which confirms this batch has passed our QC requirements (as detailed below) using vials from the same batches of components as provided in this kit.

YIELD	60-99% (depending on fragment length)
BINDING CAPACITY	40 µg of DNA
DNA PURITY A_{260}/A_{280} RATIO	1.7 – 1.9

Analysis label to be attached for each production batch

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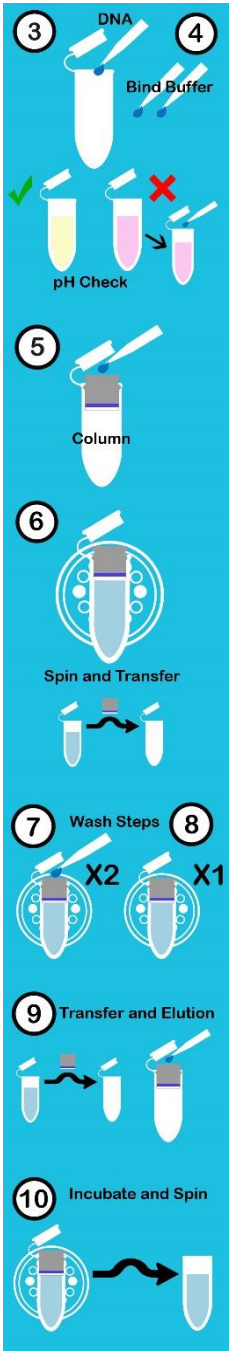
PROTOCOL

1. Prior to purification with NS Reagents DNA Clean, samples can be stored under DNase-free conditions at -20°C or -80°C (avoid multiple freeze/thaw cycles). +4°C storage is also acceptable, but ideally only for 1 or 2 days.
2. Make sure the kit buffers are mixed well (remember to add ethanol to the Wash Buffer before first use).
3. Start with up to 200 µl of DNA sample in a sterile 1.5ml tube (do not use more than 200ul as this may lead to too much volume for the column in later steps).
4. Add 2 x the DNA sample volume of Bind Buffer (so if your sample is 30ul add 60ul of Bind Buffer) and vortex for 2 seconds.
   NB If the liquid in the tube is yellow the pH is optimal for binding. If it is pink the pH is too high. Add 10ul of 3 M sodium acetate (pH 5.2) to lower the pH and bring the colour indicator back to yellow.
5. Briefly centrifuge the tube to pull down any droplets in the lid and transfer the liquid to the column and collection tube set.
6. Centrifuge for 30 seconds at 11,000g and transfer the column to a new tube, discarding the first tube and its contents.
7. Add 700ul of Wash Buffer and centrifuge for 30 seconds at 11,000g, Discard the liquid in the tube and REPEAT THIS STEP using the same tube.
8. WITHOUT adding more wash buffer centrifuge for 60 seconds at 11,000g to remove any remaining alcohol which could interfere with downstream applications if not removed.
9. Transfer the column to a sterile 1.5ml tube and add an appropriate volume of Elution Buffer. For optimal elution add the Elution Buffer precisely to the centre of the membrane. 15-30 µl is recommended, but volumes up to 200 µl can be used. The volume used should match the expected amount of DNA in the sample and the required concentration for downstream applications.
10. Incubate at room temp (15-25°C) for 1-2 minutes and then centrifuge for 60 seconds at 11,000g. To maximise retrieval, heat the Elution Buffer to 70°C before use, add the maximum 200 µl and incubate for 5 minutes or use 15-30 µl in 3 different spins incubating for 2-5 minutes each time.
11. The tube now contains your DNA and should be stored as required for your downstream application. The used column can be discarded.
12. A 6x Loading Buffer is included in the kit for analysis of the purified DNA by electrophoresis. The loading buffer contains bromophenol blue, xylene cyanol and orange G. Use 2 µl of Buffer in 10 µl of purified DNA.



TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	SUGGESTED ACTION
Low DNA yield	Ineffective DNA binding	After Bind Buffer is added, ensure mix is yellow. If it is pink, add 10 µl of 3 M sodium acetate, pH of 5.2 to correct the pH.
	No ethanol in wash buffer	Ensure 96–100% ethanol was added to Wash Buffer before use.
	Incomplete DNA elution	Before applying the Elution Buffer heat it to 70°C and extend incubation time to 5 min and/or perform second elution.
	pH of elution liquid is too low	Use Elution Buffer with a higher pH.
Purified DNA flows out of lanes in an agarose gel	Purified DNA still contains ethanol	Repeat isolation ensuring no Wash Buffer is left in the column (step 8 of protocol).
Purified DNA produces blurred bands on gel	Running buffer contains nucleases	Always use fresh buffer for electrophoresis and gel preparation.
	Storage tube contains nucleases	Always use a sterile DNase-free tube to collect your purified DNA.
	Elution solution contains DNases	Use fresh elution solution. If using water, ensure it is DNase-free.
Inhibition of downstream enzyme reactions	Running buffer contaminated	Always use fresh buffer for electrophoresis and gel preparation.
	Purified DNA contains salts	Perform all purification steps at room temperature. Ensure no sediment in Wash Buffer before use.
	Purified DNA still contains ethanol	Repeat isolation ensuring no Wash Buffer is left in the column (step 8 of protocol).
Poor DNA-Seq data	Contamination from other sources	Always use a sterile DNase-free tube to collect your purified DNA.

QUESTIONS?

If you have any questions when using any of our products, you can contact our technical team:

+44 (0)1638 551500 tech@nktscientific.com newmarketscientific.com