(Version 1.3)

DNA Gel Off - DNA isolation from agarose gels

Cat No: MB19-21010 and MB19-21050

INTENDED USE and PROTOCOL OVERVIEW

NS Reagents DNA Gel Off is for the rapid purification of DNA from agarose gels, removing other contaminants in the process. Providing up to 95% 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.

The DNA purification procedure uses spin columns which bind nucleic acids from an excised and melted fragment of agarose gel. The Bind Buffer aids gel and protein breakdown 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	MB19-21010	MB19-21050
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.
The Bind Buffer should be protected from the sunlight and all bottles should be closed tightly when not in use

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

96–100% ethanol PFA
2-propanol
1.5–2 ml microcentrifuge tubes
Microcentrifuge (≥ 11 000 x g)
Scalpel for gel cutting
3M sodium acetate, pH 5.2 (may be required)
Pipettes and tips (sterile filter tips are recommended)
Water bath/heating block up to 50°C
Standard lab PPE equipment (lab coat & gloves)
Transilluminator

SAFETY INFORMATION and PRECAUTIONS

If ethidium bromide, other harmful chemical components and UV light are to be used during gel electrophoresis and visualisation, 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
H302, H312, H332, H412
P261, P264, P270, P271, P273, P280, P301+P312 P330,
P302+P352 P312, P304+P340 P312, P363, EUH032

H302 Harmful if swallowed. **H312** Harmful in contact with skin. **H332** Harmful if inhaled. **H412** Harmful to aquatic life with long-lasting effects. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P270** Do not eat, drink or smoke when using this product. **P271** Use only outdoors or in a well-ventilated area. **P273** Avoid release to the environment. 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.

P302+P352 P31: IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/DOCTOR if you feel unwell.
P363 Wash contaminated clothing before reuse.

P304+P340 P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. EUH032 Contact with acids liberates very toxic gas.

QUALITY CONTROL, KIT SPECIFICATIONS and CERTIFICATE OF ANALYSIS

The quality of each production batch of **NS Reagents DNA Gel Off** is tested with a range of DNA fragment lengths (100 bp - 10 kb) and masses contained in agarose slices of up to 300mg. 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	70-95% (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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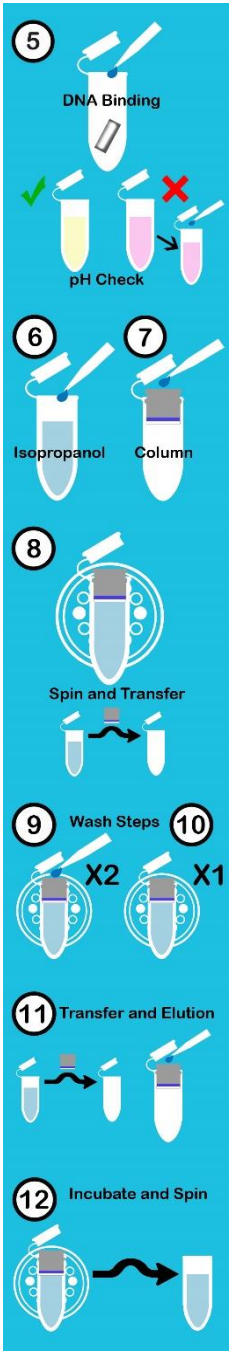
PROTOCOL

1. Carry out your gel electrophoresis using standard or low melting point agarose in either a TAE or TBE buffer until the DNA fragments of interest are sufficiently separated.
2. Cut the DNA fragment from the agarose gel using an appropriate clean cutting tool. Try to minimise the size of the gel slice. The gel slice should be 300 mg or less, larger fragments can be cut in half and run as two separate purifications.
3. Transfer the gel slice to sterile tube. Gel slices can be stored at +4°C or -20°C for up to 1 week (in DNase-free conditions).
4. Make sure the kit buffers are mixed well (remember to add ethanol to the Wash Buffer before first use).
5. Add 400 µl of Bind Buffer, mix well and incubate at 50°C in a water bath or block heater until the gel has completely dissolved (5-10 minutes). Inverting the tube a few times during this process speeds it up.
   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.
6. Add 250 µl of Isopropanol (not included in the kit) and mix well.
7. Briefly centrifuge the tube to pull down any droplets in the lid and transfer the liquid to the column and collection tube set. The column has a maximum capacity of 800ul. If you have more liquid you can simply do the next step twice.
8. Centrifuge for 30 seconds at 11,000g and transfer the column to a new tube, discarding the first tube and its contents.
9. 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.
10. 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.
11. 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. 50-100 µl is recommended but 20-200 µl can be used. The volume should match the required concentration for downstream applications.
12. Incubate at room temp (15-25°C) for at least 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.
13. The tube now contains your DNA and should be stored as required for your downstream application. The used column can be discarded.
14. 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.



Reagents

TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	SUGGESTED ACTION
Low DNA yield	Incomplete melting of gel	Use a longer 50°C incubation to ensure agarose slice is completely melted. 5 minutes extra should be enough.
	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.
Column becomes clogged during purification	Incomplete melting of gel	Use a longer 50°C incubation to ensure agarose slice is completely melted. 5 minutes extra should be enough.
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 10 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	Store gel fragment at +4°C, under DNase-free conditions (DNase-free tube), for no more than a few days.
	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 10 of protocol).
Poor DNA-Seq data	Running buffer contaminated	Always use fresh buffer for electrophoresis and gel preparation.
	DNA exposed to UV too long	Minimise DNA UV exposure during gel fragment excision.
	Contamination from other sources	Always use a clean scalpel and surface during gel excision.

QUESTIONS?

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

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