Article number:
11003313 (50 pcs.)

Version: 062018

Research use only

Axonlab LifeScience
GENOMICS

extrAXON

DNA GEL-OUT KIT

Kit for total DNA isolation from agarose gels

The **extrAXON DNA GEL-OUT KIT** is designed for a rapid and efficient purification of DNA fragments directly from agarose gels (standard and low-melting point agarose gels run in either a TAE or TBE buffer). Agarose, ethidium bromide and other contaminants from a sample are effectively removed in the purification process. The kit enables the purification of DNA fragments from 50 bp to 30 kb, as well as plasmid and genomic DNA.

Axonlab
connecting ideas

www.axonlab.com

extrAXON

DNA GEL-OUT KIT

Instructions to use
Version 062018

I. | Intended Use

The **extrAXON DNA GEL-OUT KIT** is designed for a rapid and efficient purification of DNA fragments directly from agarose gels (standard and low-melting point agarose gels run in either a TAE or TBE buffer). Agarose, ethidium bromide and other contaminants from a sample are effectively removed in the purification process. The kit enables the purification of DNA fragments from 50 bp to 30 kb, as well as plasmid and genomic DNA. However, purification of fragments smaller than 100 bp and larger than 10 kb will result in decreased recovery rates. The purified DNA can be used in common downstream applications. The purification protocol and buffer formulations were optimized for high yields and DNA purity. The product is intended for research use only.

II. | COMPONENTS OF THE KIT AND STORAGE CONDITIONS

Number of isolation	50 isolations	Storage Conditions
GB Buffer (Binding Buffer)	25 ml	15°C – 25°C in dark
GW Buffer (conc.)* (Wash Buffer)	15 ml	15°C – 25°C
Elution Buffer	10 ml	15°C – 25°C
DNA Purification Columns	50 pcs	15°C – 25°C
Collection Tubes (2 ml)	50 pcs	15°C – 25°C

* Before the first use, add an appropriate amount of **96 – 100% ethanol** to **GW Buffer** (see the instructions on the bottle label and in the table below). It is recommended to mark the bottle after adding alcohol.

Number of isolation	50 isolations
GW Buffer	15 ml
96-100% ethanol	60 ml
Total Volume	75 ml

▶ Protect the GB Buffer from the sunlight!

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

Expiry date: Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. | Additional materials and equipment required

· 96 – 100% PFA ethanol
· sterile scalpel or razor
· sterile microcentrifuge tubes (1.5 – 2 ml)
· automatic pipettes and sterile DNase-free tips
· disposable gloves
· transilluminator
· microcentrifuge with rotor for 1.5 – 2 ml (≥ 11 000 x g)
· 3 M sodium acetate, pH 5.2 (might be required)

IV. | Principle

DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. In the first step, DNA fragment is excised from an agarose gel and incubated in GB Buffer, which enables gel fragment solubilization and protein degradation. As an added convenience, binding buffer contains a color indicator, which facilitates easy monitoring of the solution's pH for optimal DNA binding. The two-step washing stage efficiently removes impurities and enzyme inhibitors. Purified DNA is eluted with the use either a low ionic strength buffer (Elution Buffer) or water (pH 7.0 – 9.0) and can be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, ligation and so forth or stored until ready to use.

V. | Quality Control

The quality of each production batch (LOT) of the **extrAXON DNA GEL-OUT KIT** is tested with the use of standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. | Product Specifications

SAMPLE MATERIAL

▶ Agarose fragment of up to 300 mg containing DNA

YIELD

70 – 95%, depending on DNA fragment length (in the range of 100 bp – 10 kb)

DNA FRAGMENT LENGTH

100 bp – 10 kb
DNA fragments in the 50 – 100 bp and 10 – 30 kbp range can also be purified, as well as genomic and plasmid DNA. However, the efficiency will be decreased.

BINDING CAPACITY

▶ Approx. 25 µg DNA

TIME REQUIRED

16 – 20 minutes

DNA PURITY

A_{260}/A_{280} ratio = 1.7 – 1.9

VII. | Safety Precautions

- If ethidium bromide or other harmful chemical components is used for gel electrophoresis image visualization, then suitable protective clothing and disposable nitrile gloves must be worn at all times.
- While excising the agarose fragment, compliance with all the safety requirements for working with UV light (protective clothing, safety goggles, nitrile disposable gloves) is essential.
- The use of sterile pipette filter tips is recommended.
- Avoid cross-contamination of DNA between minicolumns.
- Guanidine salts' can form highly reactive compounds when combined with bleach or other oxidation components. In case of spillage, clean the surface with suitable laboratory detergent and water.

VIII. | Recomendations and important notes

DNA ELUTION

An optimal volume of Elution Buffer used should be chosen in accordance with the amount of DNA in the sample and with final DNA concentration expected. The use of 30 – 100 µl of Elution Buffer is recommended.

If high DNA concentration is desired elution's volume may be reduced to 20 µl. It should be noted that this may reduce the efficiency of DNA retrieval. It is essential to apply Elution Buffer precisely to the centre of the membrane.

In order to maximize the DNA retrieval, heat Elution Buffer to 80°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, elute DNA in 200 µl of Elution Buffer or perform a second elution. For the second elution, repeat steps 11 – 14 of the Isolation Protocol (section XI), placing the purification column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions.

pH MONITORING

GB Buffer contains an indicator, which enables pH monitoring. Yellow indicates that the solution's pH is lower than 7.5, which guarantees optimal DNA binding with the membrane. When the pH is higher than 7.5, the solution turns pink. It usually happens, for example, when the running buffer for electrophoresis has been used several times or was incorrectly prepared. In such cases, it is essential to add **10 µl of 3 M sodium acetate (pH 5.2)**. It will lower the pH, enabling the solutionto bind efficiently to the minicolumn membrane.

IX. | Sample preparation

1. Conduct gel electrophoresis using standard or low melting point agarose in either a TAE or TBE buffer until DNA fragments are sufficiently separated. Using high voltage is not recommended, since this may cause an increase in buffer temperature and DNA degradation. Use freshly prepared run buffer and the buffer used for the gel preparation.
2. Weigh a sterile, 1.5 – 2 ml Eppendorf tube.
3. Excise DNA fragment from the agarose gel using a clean, sharp scalpel or razor blade. Minimize the size of the gel slice by removing any excess agarose (the weight of the agarose slice should not exceed 300 mg). The blade and transilluminator should be cleaned with a DNA remover prior to excision. As far as possible, manipulations should be carried out so as to minimize UV exposure to a few seconds. This is particularly vital when DNA isolated is to be used for sequencing or cloning.
4. Transfer the gel slice into a pre-weighed, sterile, 1.5 – 2 ml Eppendorf tube and weigh it. If the gel fragment mass exceeds 300 mg, divide it into smaller fragments and transfer them to the other 1.5 – 2 ml tube.
5. Before starting the isolation process, the gel slice containing DNA fragment may be stored at +4°C or -20°C for up to 1 week under DNase-free conditions.

X. | Prior to isolation

1. Mix well each buffer supplied with the kit.
2. Ensure that ethanol has been added to **GW Buffer**. If not, add appropriate quantity of **96 – 100% ethanol** (volumes can be found on the bottles' labels or in the table given in section II).
3. Examine all buffers. If a sediment has occurred in any of them, incubate it at **37°C (GW Buffer)** or at **50 – 60°C** (other buffers) mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Heat a dry block heater or a water bath to **50°C**.
5. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. | Isolation Protocol

1. Excise a gel slice containing the DNA fragment and place it in a 1.5–2 ml Eppendorf tube.
   - The gel slice mass should not exceed 300 mg. For instructions, see section IX. Sample preparation.

2. Add **500 µl GB Buffer** and mix well by inverting the tube for several times.

3. Incubate the mixture at **50°C for 5 – 10 minutes** or until the agarose has completely dissolved. During the incubation, mix the sample by inverting the tube several times.
   - Ensure that the agarose is completely dissolved before moving on to the next step.
   - The solution should be yellow. If it turns pink after mixing, add 10 µl of 3 M sodium acetate, pH of 5.2, and mix thoroughly (see section VIII. Recommendations and Important Notes).

4. Centrifuge the tube briefly in order to recover any remaining liquid from the lid and transfer **800 µl** of the **mixture** into a DNA Purification Column placed in a Collection Tube. Centrifuge for **60 s** at 11 000 – 15 000 x g.
   - If the volume of the mixture exceeds 800 µl in total, discard the filtrate after centrifugation, then reuse Collection Tube and transfer the remaining mixture into the same minicolumn.

5. Transfer DNA Purification Column to a new Collection Tube (2 ml).

6. Add **700 µl GW Buffer** and centrifuge for **30 s** at 11 000–15 000 x g.

7. Discard the filtrate and reuse Collection Tube.

8. Repeat steps 6 and 7.

9. Centrifuge for **60 – 120 s** at 15 000–21 000 x g.
   - GW Buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from minicolumn before elution.

10. Discard Collection Tube and filtrate and carefully transfer DNA Purification Column to a sterile, 1.5 ml Eppendorf microcentrifuge tube.

11. Add **50 µl Elution Buffer**, directly onto DNA Purification Column membrane.
    - Other buffer volumes in the 20 – 200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

12. Incubate DNA Purification Column at room temperature for **120 s**.

13. Centrifuge at 11 000 – 15 000 x g for **60 s**.

14. Remove DNA Purification Column. Isolated DNA should be stored at **+4°C or -20°C** depending on further applications.

XII. | Troubleshooting

Problem	Possible cause	Solution
Low yield of purified DNA.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely lysed. After lysis, incubate sample for an additional 5 minutes.
	Ineffective DNA binding to membrane.	Ensure that mixture is yellow after adding GB Buffer. If the color turns pink, add 10 µl of 3 M sodium acetate, pH 5.2.
	Ethanol was not added to GW Buffer.	Ensure that 96 – 100% ethanol was added to GW Buffer before use.
	Incomplete DNA elution from membrane.	Before applying Elution Buffer to the membrane, heat it to 80°C. Apply Elution Buffer directly to centre of the membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution.
	pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
Column becomes clogged during purification.	Incomplete agarose slice dissolution.	Extend incubation at 50°C until agarose slice is completely lysed. After lysis, incubate sample for an additional 5 minutes.
DNA flows out of the lanes in agarose gel.	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether any residual GW Buffer is left in DNA Purification Column after centrifugation in step 9.
Blurred bands in gel electrophoresis image.	Running buffer contains nucleases.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
		Store the gel fragment at +4°C, under DNase-free conditions, for no more than a few days.
	Elution solution contains DNases.	Use fresh elution solution. If water is used instead of Elution Buffer, ensure that it is DNase-free.

Problem	Possible cause	Solution
Inhibition of downstream enzymatic reactions.	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
	Purified DNA contains residual salts.	Perform all centrifugation steps at room temperature. Ensure that there is no sediment in GW Buffer before use.
	Purified DNA contains residual alcohol.	Repeat isolation, paying a particular attention to whether any residual GW Buffer is left in DNA Purification Column after centrifugation in step 9.
Incorrect DNA sequencing results.	Running buffer for electrophoresis was contaminated.	Always use freshly prepared buffer for both electrophoresis run and gel preparation.
	Extensive exposure to UV light.	Minimize the DNA's exposure time to UV light during excision from gel procedure.
	Equipment has been contaminated.	Clean scalpel or razor blade and transilluminator surface prior to gel slice excision.

XIII. | Safety information

extrAXON

DNA GEL-OUT KIT

GB Buffer

H302, H312, H332, H412
P273, P301+P312 P330, P304+P340
P312, EUH032

Warning

EUH032 Contact with acids liberates very toxic gas. **H302** Harmful if swallowed. **H312** Harmful in contact with skin. **H332** Harmful if inhaled. **H412** Harmful to aquatic life with long-lasting effects. **P273** Avoid release to the environment. **P301+P312 P330** IF SWALLOWED: Call a POISON CENTER / doctor if you feel unwell. Rinse mouth. **P304+P340 P312** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a P OISON CENTER / doctor if you feel unwell.

10904A 042018

Axon Lab AG
Täfernstrasse 15
CH-5405 Baden-Dättwil
info@axonlab.ch
www.axonlab.com

Axonlab

connecting ideas