New protocol 19.03.2012 Gel extraction

INSTRUCTION MANUAL

Zymoclean™ Gel DNA Recovery Kit

Catalog Nos. **D4001, D4002, D4007 & D4008**

Highlights

- Quick (15 minute) recovery of ultra-pure DNA from agarose gels.
- Column design permits DNA elution at high concentrations into minimal volumes (≥6 µl).
- Eluted DNA is well suited for use in DNA ligation, sequencing, labeling, PCR, *etc*.

Contents

Product Contents

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Zymoclean™ Gel DNA Recovery Kit

(Kit Size)	**D4001, D4007** (50 preps.)	**D4002, D4008** (200 preps.)	**Storage Temperature**
ADB	50 ml	2x100 ml	Room Temp.
DNA Wash Buffer[1]	6 ml	24 ml	Room Temp.
Zymo-Spin™ I Columns	50 ct. D4001 – w/ uncapped columns D4007 – w/ capped columns	200 ct. D4002 – w/ uncapped columns D4008 – w/ capped columns	Room Temp.
Collection Tubes	50	200	Room Temp.
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Buffer Preparation

Before starting, add 24 ml 100% ethanol to the 6 ml **DNA Wash Buffer** concentrate (96 ml 100% ethanol to the 24 ml **DNA Wash Buffer** concentrate) to obtain the final **DNA Wash Buffer** solution. Alternatively, add 26 ml and 104 ml of 95% ethanol to the 6 ml and 24 ml sizes of the **DNA Wash Buffer** concentrate, respectively.

Protocol

1. Excise the DNA fragment[1] from the agarose gel using a razor blade or scalpel and transfer it to a 1.5 ml microcentrifuge tube.
2. Add 3 volumes of **ADB** to each volume of agarose excised from the gel (*e.g.* for 100 µl (mg) of agarose gel slice add 300 µl of **ADB)**.
3. Incubate at 37-55 °C for 5-10 minutes until the gel slice is completely dissolved[2].

   For DNA fragments >8 kb, following the incubation step, add one additional volume (equal to that of the gel slice) of water to the mixture for better DNA recovery (*e.g.* 100 µl agarose, 300 µl **ADB** and 100 µl water).
4. Transfer the melted agarose solution to a **Zymo-Spin™ Column** in a **Collection Tube**.
5. Centrifuge at ≥10,000 x *g* for 30-60 seconds. Discard the flow-through.
6. Add 200 µl of **Wash Buffer** to the column and centrifuge at ≥10,000 x *g* for 30 seconds. Discard the flow-through. Repeat the wash step.
7. Add ≥6 µl of water[3,4] directly to the column matrix. Place column into a 1.5 ml tube and centrifuge ≥10,000 x *g* for 30-60 seconds to elute DNA.

   Ultra-pure DNA in water is now ready for use.

tes:

The amount of agarose cised from the gel should as small as possible.

Do not incubate above °C. It is important that gel slice dissolves mpletely. This can be ilitated by gently mixing ring the incubation.

Elution of DNA from the lumn is dependent on pH d temperature. If water is ed, make sure the pH >5.0. Waiting 1 minute er adding water to the lumn may improve the ld of larger (> 6 kb) DNA. r even larger DNA (> 10) the total yield may be proved by eluting the DNA th 60-70 °C water.

TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) or odified TE (10 mM Tris, 1 mM EDTA, pH 8.5) can so be used for elution if quired by your experiment.

Please find the detailed protocol in the extraction kit in the lab or on the following link:

http://www.zymoresearch.com/dna-purification/dna-clean-up/gel-dna-recovery/zymoclean-gel-dna-recovery-kit

old protocol from Bonn:

QIAEX II Agarose Gel Extraction Protocol

This protocol is designed for the extraction of 40-bp to 50-kb DNA fragments from 0.3–2% standard or low-melt agarose gels in TAE or TBE buffers.

Notes:
- The yellow color of Buffer QX1 indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- A heating block or water bath at 50°C is required.
- 3M sodium acetate, pH 5.0, may be necessary.
- All centrifugation steps are at maximum speed (≥10,000 x *g*, ~13,000 rpm) in a conventional, table-top microcentrifuge.
- For DNA fragments larger than 10 kb, mix by gently flicking the tube to avoid shearing the DNA. Do not vortex the tube.

1. **Excise the DNA band from the agarose gel with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing excess agarose. Use a 1.5-ml microfuge tube for processing up to 250 mg agarose.

2. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QX1 to 1 volume of gel for DNA fragments 100 bp – 4 kb; otherwise, follow the table below.**
   For example, add 300 µl of Buffer QX1 to each 100 mg of gel.

DNA fragments <100 bp	Add 6 volumes of Buffer QX1
DNA fragments >4 kb	Add 3 volumes of Buffer QX1 plus 2 volumes of H_2O
>2% or Metaphor agarose gels	Add 6 volumes of Buffer QX1

3. **Resuspend QIAEX II by vortexing for 30 sec. Add QIAEX II to the sample according to the table below and mix.**

≤2 µg DNA	Add 10 µl of QIAEX II
2–10 µg DNA	Add 30 µl of QIAEX II
Each additional 10 µg DNA	Add additional 30 µl of QIAEX II

4. **Incubate at 50°C for 10 min to solubilize the agarose and bind the DNA. Mix by vortexing* every 2 min to keep QIAEX II in suspension. Check that the color of the mixture is yellow.**
   If the color of the mixture is orange or purple, add 10 µl 3M sodium acetate, pH 5.0, and mix. The color should turn to yellow. The incubation should then be continued for an additional 5 min at least.

The adsorption of DNA to QIAEX II particles is only efficient at pH ≤7.5. Buffer QX1 now contains a pH indicator which is yellow at pH ≤7.5, and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. **Centrifuge the sample for 30 sec and carefully remove supernatant with a pipet.**

6. **Wash the pellet with 500 µl of Buffer QX1.**
   Resuspend the pellet by vortexing*. Centrifuge the sample for 30 sec and remove all traces of supernatant with a pipet. This wash step removes residual agarose contaminants.

7. **Wash the pellet twice with 500 µl of Buffer PE.**
   Resuspend the pellet by vortexing*. Centrifuge the sample for 30 sec and carefully remove all traces of supernatant with a pipet. These washing steps remove residual salt contaminants.

8. **Air-dry the pellet for 10–15 min or until the pellet becomes white.**
   If 30 µl of QIAEX II suspension is used, air-dry the pellet for approximately 30 min. Do not vacuum dry, as this may cause overdrying. Overdrying the QIAEX II pellet may result in decreased elution efficiency.

9. **To elute DNA, add 20 µl of 10 mM Tris·Cl, pH 8.5 or H_2O and resuspend the pellet by vortexing*. Incubate according to the table below.**

DNA fragments ≤4 kb	Incubate at room temp. for 5 min
DNA fragments 4–10 kb	Incubate at 50°C for 5 min
DNA fragments >10 kb	Incubate at 50°C for 10 min

   Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) but the EDTA may inhibit subsequent enzymatic reactions.

10. **Centrifuge for 30 sec. Carefully pipet the supernatant into a clean tube.**
    The supernatant now contains the purified DNA.

11. **Optional: repeat steps 9 and 10 and combine the eluates.**
    A second elution step will increase the yield by approximately 10–15%.

* *For fragments larger than 10 kb, resuspend the pellet by inverting and flicking the tube. Vortexing can cause shearing of large DNA fragments.*

Änderung 01/07(CW): wichtig:alle Zentrifugationen 2min bei 14000rpm bei Rt durchführen!Pellet nach letztem Waschschritt nicht

vollständig trocknen ,sondern nur solange bis es leicht weiß ist!Nach Elution der DNA in 20µl bid , diese für 10 min.bei 65°C mit offenem Eppideckel inkubieren! Anschließend ein Aliquot auf Minigel auftragen!

Elution von DNA- Fragmenten aus Low Melting- Agarose mit beta-Agarase

Gut geeignet für sehr große Fragmente (größer 15 Kb)

- Funktioniert nur mit Low Melting-Agarose (LMA)!!!

- DNA-haltiges Gelstückchen so eng wie möglich ausschneiden und einmal mit 1 x beta-Agarase-Puffer waschen.

- 1/10 Volumen 10 x beta-Agarase-Puffer zugeben und 10 –15 min bei 65° C inkubieren (Agarose muß vollständig geschmolzen sein). Auf 40° C abkühlen.

- 1 µl (1 u) beta-Agarase pro 200 µl 1 % LMA-Gel zugeben und mischen. 1 h bei 40° C inkubieren.

- 1/10 Vol. 3 M NaOAc zugeben, mischen und 15 min auf Eis inkubieren. 10 min bei 14 000 rpm abzentrifugieren. DNA-haltigen Überstand in neues Eppi überführen.

- 1 µl Glykogen und 2 Vol. EtOH zugeben, mischen und wie zuvor zentrifugieren. Überstand verwerfen, Pellet einmal mit etwa 400 µl 70 % EtOH waschen. Lufttrocknen und in TE resuspendieren.