DATA SHEET

Jena Bioscience

Agarose Gel Extraction Kit - Column Kit

Spin-column based DNA cleanup from agarose gels

Cat. No.	Amount
PP-202S	50 preparations
PP-202L	250 preparations

For *in vitro* use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Shelf Life: 12 months

Description:
Agarose Gel Extraction Kit is designed for high-yield recovery of DNA from agarose gel with simultaneous removal of primer-dimers, primers, nucleotides, proteins, salt, agarose, ethidium bromide, and other impurities. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. The kit provides a simple and efficient way to purify DNA in a size range between 100 bp and 10 kb. It requires no organic extractions or precipitation and guarantees high and stable recovery rates.

Content:
Extraction Buffer
Activation Buffer
Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)
Elution Buffer
Spin Columns
2 ml Collection Tubes

To be provided by you:
96-99 % Ethanol
Isopropanol
1.5 ml microtubes

Preparation procedure:
The agarose gel is dissolved in the chaotropic Extraction Buffer followed by a simple binding, washing, and eluting procedure. Before start, add 96-99 % Ethanol to the Washing Buffer as indicated on the bottle.

Buffer	**PP-202S** 50 preps	**PP-202L** 250 preps
Extraction Buffer	75 ml	2x 185 ml
Activation Buffer	6 ml	30 ml
Washing Buffer	add 64 ml Ethanol (final volume 80 ml)	add 160 ml Ethanol to each bottle (final volume 200 ml each)
Elution Buffer	5 ml	25 ml

The additional use of Isopropanol enhances yield and is recommended for fragments smaller than 200 bp or larger than 5 kbp. The optional secondary washing step minimizes the salt content of the purification product but may significantly reduce the yield of DNA fragments <200 bp.

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1 Excision of the Gel:

- Cut the area of gel containing the DNA fragment.
- Transfer the excised gel to a clean 1.5 ml microtube.

2 Sample Preparation:

- Add 3 volumes of Extraction Buffer to 1 volume of the sliced gel. For example, add 300 µl Extraction Buffer to each 100 mg (approx. 100 µl) gel. For gels containing >2.5 % agarose, add 6 volumes of Extraction Buffer per gel volume.
- Incubate at 60 °C for 10 min with occasional mixing to ensure gel dissolution.
- Add 1 volume Isopropanol per gel volume to the dissolved gel and mix well.
- For purification of DNA fragment sizes smaller than 200 bp or larger than 5 kbp increase the amount of Isopropanol to 2 volumes.

3 Column Activation:

- Place a Spin Column into a 2 ml collection tube.
- Add 100 µl of Activation Buffer into the Spin Column.
- Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.

4 Column Loading:

- Apply the sample mixture from step 2 into the activated Spin Column.
- Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.
- Discard the flow-through.

5 Column Washing:

- Place the DNA loaded Spin Colum into the used 2 ml tube.
- Apply 700 µl of Washing Buffer to the Spin Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.

Optional Secondary Washing: Recommended only for DNA >200 bp and if highly purified DNA for DNA sequencing, transfection etc. is required.

- Add 700 µl of Washing Buffer to the Spin Column.
- Centrifuge at 10,000 g for 30 sec and discard the flow-through.

- Centrifuge again for 2 min to remove residual Washing Buffer.

6 Elution:

- Place the Spin Column into a clean 1.5 ml microtube (not provided in the kit).
- Add 30-50 µl Elution Buffer or dd-water to the center of the column membrane.
- Incubate for 1 min at room temperature.
- Centrifuge at 10,000 g for 1 min to elute DNA.