

Agarose Gel Extraction Kit

DNA Extraction by silica-gel membrane adsorption

DNA Cleanup

	Cat.-No.	Amount
	PP-202XS	10 preparations
	PP-202S	50 preparations
	PP-202L	250 preparations

For *in vitro* use only
 Quality guaranteed for 12 months
 Store at room temperature

Kit contents

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

To be provided by you

96-99% Ethanol
 Isopropanol (optional)
 1.5 ml microtubes

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.

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-202XS	PP-202S	PP-202L
	10 preps	50 preps	250 preps
Extraction Buffer	15 ml	75 ml	2x 185 ml
Activation Buffer	1.2 ml	6 ml	30 ml
Washing Buffer	add 12 ml Ethanol (final volume 15 ml)	add 64 ml Ethanol (final volume 80 ml)	add 160 ml Ethanol to each (final volume 200 ml each)
Elution Buffer	1.2 ml	6 ml	30 ml

The additional use of Isopropanol (not included in the kit) 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.

Agarose Gel Extraction Kit

DNA Extraction by silica-gel membrane adsorption

DNA Cleanup

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.
- For DNA fragment sizes smaller than 200 bp or larger than 5 kbp and to enhance yield add 1 volume *Isopropanol* per gel volume to the dissolved gel and mix well.

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 Column* 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, 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.