Lumiprobe
Life science solutions

LumiSpin® GEL, DNA Isolation Spin Kit for Agarose Gel manual

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The kit allows rapid (about 20 minutes) and efficient ($OD_{260}/OD_{280} > 1.8$) purification of DNA fragments from agarose gels or enzymatic reactions. Spin columns supplied with the kit enable purification of up to 5 or 20 µg of DNA 70 bp–10 kb in size, and the recovery of the purified DNA is not less than 50 % when purifying from agarose gels and not less than 75 % when purifying from enzymatic reactions. The purified DNA is suitable for any subsequent application, such as PCR, restriction enzyme digestion, ligation and transformation, labeling, Southern blotting, sample preparation for Sanger sequencing and NGS, etc.

Kit components

Kit component	Count			
	13793 10 preps (5 µg)	23793 50 preps (5 µg)	15793 10 preps (20 µg)	25793 50 preps (20 µg)
11164, Spin column (up to 5 µg), 10 pcs	1	—	—	—
21164, Spin column (up to 5 µg), 50 pcs	—	1	—	—
12164, Spin column (up to 20 µg), 10 pcs	—	—	1	—
22164, Spin column (up to 20 µg), 50 pcs	—	—	—	1
D1450, Neutralization Buffer, 2 mL	1	1	1	1
K3450, Gel Solubilization Buffer, 10 mL	1	—	1	—
K2250, Wash Solution B (Concentrate to be diluted 5x with ethanol 96%), 10.0 mL	1	1	1	1
D1350, Elution Buffer (10 mM Tris-HCl, pH 8.5), 1.5 mL	1	2	1	4
Collection tube for spin column, 2 mL	10	50	10	50
S3450, Gel Solubilization Buffer, 50 mL	—	1	—	1

Store and transport at room temperature.

Shelf life 12 months.

Equipment and reagents supplied by user:

- 96 % ethanol (the necessary volume is equal to 4 volumes of the *Wash Solution B* concentrate supplied with the kit)
- isopropanol (about 100 µL for DNA purification from 1 typical sample)
- 1.5 mL microcentrifuge tubes (2 tubes for DNA purification from 1 sample)
- microcentrifuge with rotor for 1.5 mL tubes and capable of centrifuging >10,000 RPM (6,700 × *g*)
- heating block (alternatively, a water bath can be used)
- laboratory balance with a precision of at least 0.01 g

Before starting

1. Dilute the *Wash Solution B* concentrate 5× with 96 % ethanol (add 4 volumes of 96 % ethanol to 1 volume of concentrate specified on the bottle). Mark on the label that ethanol has been added.
2. If any precipitate has formed in *Gel Solubilization Buffer*, incubate the solution at a temperature not higher than 50 °C until the precipitate has been completely dissolved.

DNA fragment extraction from the gel

1. Set a heating block to 50 °C.
2. Excise a gel slice containing the DNA fragment with a clean scalpel or razor blade. Minimize the size of the gel slice by cutting as close to the DNA as possible.

   ! Reduce UV exposure of the gel slice containing the DNA fragment or keep the

gel slice on a glass or plastic plate during UV illumination. This minimizes DNA damage induced by UV light.

3. Weigh a clean 1.5 mL microcentrifuge tube and tare the balance. Place the gel slice into an equivalent clean 1.5 mL tube and weigh. Record the weight of the gel slice.
4. Add 4 µL of *Gel Solubilization Buffer* to every mg of agarose gel (4+1) (e.g., add 400 µL of Gel Solubilization Buffer to a gel slice with a weight of 100 mg).

   ! When working with multiple samples — gel slices containing DNA fragments — it is convenient to add to all samples the same volume of Gel Solubilization Buffer calculated for the biggest gel slice.
5. Incubate the tube at 50 °C for 10 min with occasional mixing by inversion (1–2 times) until the gel slice is completely dissolved.

   ! For gels with an agarose content greater than 2 %, increase the incubation time until the gel slice has completely dissolved.
6. After the gel slice has dissolved completely, check that the color of the mixture is yellow. If the color of the mixture is violet, add 5 µL of *Neutralization Buffer* to every 1000 µL of the DNA sample. The color of the mixture will turn to yellow.

   The Gel Solubilization Buffer contains a color pH indicator allowing easy monitoring of the pH value optimal for DNA binding to the column membrane. Violet color indicates that the solution is too alkaline. In this case Neutralization Buffer should be added to adjust the pH value for efficient DNA binding.
7. Allow the contents of all tubes to cool down to room temperature.
8. Add 1 µL of isopropanol to every mg of solubilized agarose gel (e.g., add 100 µL of isopropanol to 100 mg solubilized gel slice). Mix thoroughly.

DNA purification

All centrifugation steps are carried out at room temperature, at 10,000–13,000 RPM (6,700–11,000 x *g*).

1. Place a spin column in a collection tube. Add the sample to the column (to a maximum of 900 µL per single load). Centrifuge the column for 30 seconds. Discard the flow-through and place the column back into the same collection tube. For sample volumes exceeding 900 µL load the remainder and repeat the procedure.
2. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the flow-through and place the column back into the same collection tube.
3. *(optional)* Repeat the washing. Add 500 µL of *Wash Solution B*, centrifuge the column for 3 minutes. Discard the collection tube with the flow-through.
4. Place the spin column into a clean 1.5 mL microcentrifuge tube. Add *Elution Buffer* to the center of the spin column membrane: 10–50 µL when using spin column with binding capacity of 5 µg DNA or 25–100 µL when using spin column with binding capacity of 20 µg DNA. Incubate at room temperature for 1 minute. Centrifuge the column for 1 minute. The microcentrifuge tube contains the purified DNA.

   ! For increased DNA concentrations, use a low volume of Elution Buffer. Elution with volumes of less than 10 µL (spin column with binding capacity of 5 µg DNA) or 25 µL (spin column with binding capacity of 20 µg DNA) is not recommended because this can be insufficient to entirely wet the membrane resulting in low DNA recovery. For increased DNA yield, use a higher volume of Elution Buffer (50 µL when using spin column with binding capacity of 5 µg DNA or 100 µL when using spin column with binding capacity of 20 µg DNA).

   ! If necessary, the DNA can be eluted with deionized water.

DNA fragment purification from enzymatic reactions

1. Add 4 volumes of *Gel Solubilization Buffer* to 1 volume of the reaction sample (e.g., add 400 µL of Gel Solubilization Buffer to 100 µL of the reaction sample). Mix thoroughly.
2. Check that the color of the mixture is yellow. If the color of the mixture is violet, add 5 µL of *Neutralization Buffer* to every 1000 µL of the DNA sample. The color

of the mixture will turn to yellow.

The Gel Solubilization Buffer contains a color pH indicator allowing easy monitoring of the pH value optimal for DNA binding to the column membrane. Violet color indicates that the solution is too alkaline. In this case Neutralization Buffer should be added to adjust the pH value for efficient DNA binding.

3. Add 1 volume of isopropanol to 1 volume of the initial reaction solution (e.g., add 100 µL of isopropanol to 100 µL of the initial reaction solution that was mixed with 400 µL of *Gel Solubilization Buffer*). Mix thoroughly.
4. Proceed with the protocol «DNA purification» above.

Note

When measuring the DNA concentration, dilute the sample only with TE buffer pH 8.5 or *Elution Buffer* supplied with the kit. Otherwise, DNA concentration and purity (OD_{260} and OD_{260}/OD_{280}, respectively) can be estimated incorrectly.

Storage of purified DNA: for long-term storage -20 °C, for short-term storage 4 °C.

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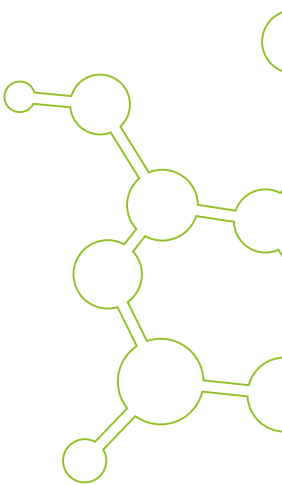

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