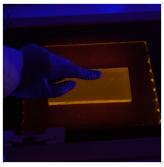

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Agarose Gel DNA Extraction (QIAquick)

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In Development This protocol is published without a DOI.

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ABSTRACT

Extract DNA from agarose gel using Qiagen® QIAquick® Gel Extraction Kit. The kit can be used for centrifuge processing or vacuum processing. The steps given here are only for centrifuge processing.

Link to the original product website is provided in the Exernal Link field. PDF file of full protocol from manufacturer is attached (available online). I do not claim any credit for the development of the kit or protocol. Please refer to the original protocol provided by Qiagen for any clarifications.

EXTERNAL LINK

https://www.qiagen.com/us/products/discovery-translational-research/dna-rna-purification/dna-purification/dna-clean-up/qiaquick-gel-extraction-kit/#orderinginformation

ATTACHMENTS

QIAquick_spin_handbook.pdf

PROTOCOL CITATION

Kenneth Schackart 2020. Agarose Gel DNA Extraction (QIAquick). **protocols.io** https://protocols.io/view/agarose-gel-dna-extraction-qiaquick-bc9yiz7w

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https://www.qiagen.com/us/products/discovery-translational-research/dna-rna-purification/dna-purification/dna-clean-up/qiaquick-gel-extraction-kit/#orderinginformation

KEYWORDS

gel extraction, Qiagen, QIAquick, DNA extraction, DNA purification

IMAGE ATTRIBUTION

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GUIDELINES

This protocol is for the purification of up to **10 µg** DNA of length 70 bp - 10 kbp.

Add 96-100% ethanol to buffer PE before use.
All centrifugation steps are carried out at **17900 x g** or the highest setting if your centrifuge does not support this speed.

MATERIALS

NAME	CATALOG #	VENDOR
Qiagen QIAquick Gel Extraction Kit		

MATERIALS TEXT

- **[M]95 % volume** - **[M]100 % volume** Isopropanol (IPA).
- 1.5 mL centrifuge tube(s)
- Nuclease free water
- Ethanol for suspending Buffer PE before use

SAFETY WARNINGS

- Gloves must be worn at all times.
- Wear proper PPE when excising gel band if using a UV light source for fluorescence. (In our lab we wear a lab coat, gloves, and polycarbonate full-face mask).
- Acute signs of UV exposure damage include redness and inflammation (like a sunburn), take note of this and address any deficiency in PPE.
- Long-term damage due to UV exposure includes increased chance of skin cancer and eyesight damage.

DISCLAIMER:

BEFORE STARTING

- Run gel electrophoresis to generate DNA band(s).
- Set heat block to **50 °C** .
- Obtain **[M]95 % volume** - **[M]100 % volume** Isopropanol (IPA).

Excise Gel

1 Label and weigh 1 uncolored 1.5 mL centrifuge tube for each gel band to be exracted.

2 Using a clean razor blade, excise DNA band from gel and place in corresponding 1.5 mL centrifuge tube. Try to cut the gel as close to the DNA band as possible

It is critical to avoid exposure of skin or eyes to UV irradiation. When using UV light source for fluorescence ensure that all skin is covered. Wear a full-face mask made of a UV blocking material such as polycarbonate. Be aware of others around you to avoid accidentally exposing them to UV irradiation.

It may be best to use a dulled razor blade when cutting to avoid damage to transilluminator surface.

3 Weigh the 1.5 mL tube with the DNA gel slice, and determine weight of gel.

Subtract the tube weight found in step 1 to detemine gel weight.

4 Add 3 volumes Buffer QG to 1 volume gel. For >2% agarose gels, add 6 volumes Buffer QG instead of 3.

100 mg gel ~ **100 µl**

Dissolve Gel

5 Incubate at **50 °C** for **00:10:00** or until gel has completely dissolved.

5.1 Vortex every 2-3 min to help dissolve gel.

6 After gel slice has dissolved, check the color.
- If color is yellow, move to next step.
- If color is orange or violet: add **10 µl** **3 Molarity (M) sodium acetate** , **pH 5** , and mix.

7 Add 1 volume isopropanol to the tube and vortex mix.

Bind and Spin DNA

8 Place a QIAquick spin column in a provided 2 mL collection tube.

9 Apply the sample to the QIAquick column and centrifuge for **00:01:00** .

If volume exceeds **800 µl** , add and centrifuge only **800 µl** first, then add and centrifuge the remaining volume.

10 Discard flow-through and place the QIAquick column back into the same tube.

11 Add **500 µl** Buffer QG to the QIAquick column and centrifuge for **00:01:00** . Discard flow-through and place back into the same tube.

Wash DNA

12 Add **750 µl** Buffer PE to QIAquick column and centrifuge for **00:01:00** . Discard flow-through and place column back into the same tube.

Elute DNA

13 Place QIAquick column into a clean 1.5 mL centrifuge tube.

14 Add **50 µl** Buffer EB or Nuclease Free Water to the center of the QIAquick membrane.

For increased DNA concentration add **30 µl** instead.

15 Let stand for **00:04:00** to increase DNA yield.

16 Centrifuge the column for **00:01:00** .

17 Discard the QIAquick column, and store DNA in microcentrifuge tube or aliquot to smaller volume.