User Guide

Montage® DNA Gel Extraction Kit

DNA Extraction From Agarose Gels, Range: 100-10,000 bp DNA

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

LUXGCS

Introduction

One of the most useful techniques in functional genomics labs is the ability to separate a number of DNA fragments by electrophoresis through an agarose gel, then extract one or more individual DNA fragments from agarose gel slices for subsequent applications such as cloning, sequencing, or radioisotopic labeling.

The Montage® DNA Gel Extraction Kit contains a centrifugal filter device (Montage® DNA Gel Extraction Device) designed to extract DNA fragments that are 100 to 10,000 bp in size from agarose gel slices in one 10-minute spin. In addition, the kit contains a modified TAE buffer that allows the casting and running of the gel from which the DNA fragment is to be extracted. The modified TAE buffer is formulated with a lower concentration of EDTA than standard TAE in order to minimize any interference by the EDTA on enzyme activity required for downstream applications.

DNA extracted with this kit requires no further purification for use in cloning and radioisotopic or fluorescent DNA sequencing. Due to the high resolving power of agarose gel electrophoresis, the small and large non-specific amplification products that frequently interfere with cloning and sequencing after PCR are completely removed from your product.

Kit Components

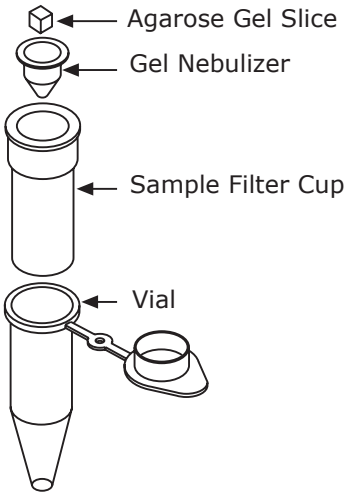
- Pre-assembled Montage® DNA Gel Extraction three-part device: Gel nebulizer, sample filter cup (0.45 µm Durapore® filter), and microcentrifuge filtrate vials, 50 units
- 50× modified TAE buffer concentrate in 500 mL bottle. Ready to dilute to 1x and use, 1 bottle
- 50× concentration = 2 M Tris-acetate,
- 5 mM Na_2EDTA, pH 8.0

Additional Materials Required

SeaKem® LE agarose or equivalent.

Procedure for Extracting DNA from Agarose Gels

1. Electrophorese the DNA of interest (restriction digest or PCR product or other DNA) through a < 1.25% agarose gel (containing 0.5 µg/mL ethidium bromide) that is cast and run in 1× Modified TAE buffer (prepared from the stock 50× described above).
2. Locate the DNA band of interest in the gel using a long-wavelength UV lamp or a transilluminator. Cut and excise the gel slice containing the DNA. The gel slice must be under 100 µL in volume or 100 mg in mass.
3. Make sure the Montage® DNA Gel Extraction Device is assembled as follows:



4. Place the gel slice in the Gel Nebulizer and seal the device with the cap attached to the vial.
5. Spin the assembled device for 10 minutes at 5,000 × *g*. Centrifugation forces the agarose through the Gel Nebulizer, converting it into a fine slurry that is captured by the Sample Filter Cup. The extruded DNA passes (in TAE buffer) through the microporous membrane in the Sample Filter Cup and is collected in the filtrate vial.
6. Discard the Sample Filter Cup and Gel Nebulizer units. The purified DNA in the vial is now ready for sequencing, radiaolabeling, or cloning procedures without further purification.

Buffer Dilution

The enclosed 50× concentrate of modified TAE buffer is ready for dilution and use.

To dilute to a 1× working solution, add 1 part of the 50× concentrate to 49 parts of nuclease-free water.

The concentration of the diluted 1× working solution is 40 mM Tris-Acetate, pH 8.0, 0.1 mM Na_2EDTA.

Notes:
Low melting point agarose is not compatible with this protocol.

We recommend modified TAE rather than TBE for the following reasons:

- TBE buffer strongly inhibits DNA sequencing reactions while modified TAE buffer does not.
- Modified TAE has 0.1 mM Na_2EDTA while standard TAE has 1.0 mM Na_2EDTA. A 0.1 mM Na_2EDTA will not interfere with the magnesium concentration required for downstream enzymatic reactions.

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