User Guide

Ultrafree®-DA

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

42600

Introduction

This centrifugal filter device is designed to extract 100 to 10,000 bp DNA from agarose gel slices in one 10-minute spin. DNA prepared with this device 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.

Contents of Package	Quantity/pk
Pre-assembled Ultrafree®-DA with: Gel Nebulizer, Ultrafree®-MC (0.45 µm Durapore®) and Microcentrifuge filtrate vials	50

Additional Materials Required

- Modified TAE* electrophoresis buffer (40 mM Tris-acetate, pH 8.0, 0.1 mM Na_2EDTA).
- SeaKem® LE agarose (FMC BioProducts, Rockland, ME) or equivalent.

Note: 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 regular TAE has 1.0 mM Na_2EDTA. The EDTA level at 0.1 mM Na_2EDTA will not interfere with the magnesium concentration in sequencing reactions and other downstream enzymatic treatments, many that are dependent on magnesium.

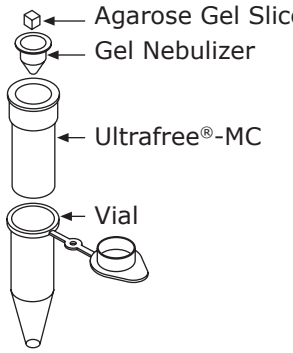
Procedure to Use Ultrafree-DA

1. Electrophorese 30 µL of PCR product or other DNA through a < 1.25% ordinary agarose gel, prepared in modified TAE buffer with ethidium bromide (0.5 µg/mL).

   Note: For best results, use modified TAE electrophoresis buffer (not TBE or normal TAE).

2. Locate band of interest with the long-wavelength ultraviolet lamp or transilluminator. With a razor blade, cut out the slice of agarose (< 100 µL at 100 mg) containing the band of interest.

   The Ultrafree®-DA is pre-assembled as follows:



3. Place gel slice into Gel Nebulizer and seal device with the cap attached to vial.
4. Spin at 5,000 x *g* for 10 minutes. Centrifugation forces the agarose through the Gel Nebulizer, converting it to a fine slurry that is captured by Ultrafree®-MC. Extruded DNA in electrophoresis buffer passes through the microporous membrane in Ultrafree®-MC and collects in the filtrate vial.
5. Discard the Ultrafree®-MC and Gel Nebulizer. You can sequence or clone the DNA in the filtrate without further purification.

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