Product No.	Product Name	Description
D6493-01/02 D6492-01/02	Cycle-Pure Kit	PCR product purification, Q-Column format & V-column format.
D1043-01/02	E-Z 96 Cycle-Pure Kit	96 well format PCR purification
D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Extraction Kit	Isolate DNA from polyacrylamide gel
D2501-01/02 D2500-01/02	Gel Extraction Kit	Agarose gel extraction using spin column technology
D2510-01/02	Ultra-Sep Gel Purification Kit	Agarose gel extraction using silica beads.
R6376-01/02	Poly-Gel RNA Purification Kit	Isolate RNA from polyacrylamide gel
R6249-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format
D6293-01/02	MicroElute Cycle Pure Kit	PCR product purification, MicroElute Spin colume
D6294-01/02	MicroElute Gel Extraction Kit	Agarose gel extraction using MicroElute spin column technology
D6296-01/02	MicroElute DNA clean Up Kit	DNA product purification with MicroElute Spin column.
D6274-01/02	MicroElute RNA Clean Up Kit	RNA product purification with MicroElute Spin column.
M1322-01/02	MagBind Cycle Pure Kit	PCR product purification with magnetic technology
M1320-01/02	MagBind Dye removal Kit	remove Dye terminator with magnetic technology.
S5912-01/02	Ultra-Sep Dye removal Kit	remove Dye terminator with column.

For technical support or to place orders, contact Omega Bio-Tek:
Tel: 800-832-8896 (toll free), 770-931-8400 (local/international)
Fax: 888-624-1688 (toll free), 770-931-0230 (local/international)
E-mail: info@omegabiotek.com

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Refer to label on bottle for directions.

7. Discard liquid and completely remove the liquid from microcentrifuge tube. Air-dry the pellet for 10-15 minutes. *This step is critical for good DNA yields.*

8. **Add 15-50 µl (depending on desired concentration of final product) Elution Buffer (10mM Tris, pH 8.5) to the tube**. Resuspend the pellet by vortexing. Incubate at 50°C in a water bath for 5 minutes. Centrifuge for 1 min at 10,000 x g to pellet the Ultra-Sep® beads .

9. Carefully transfer the supernatant to a clean tube. The supernatant now contains pure DNA. This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

   Note: efficiency of eluting DNA from Ultra-Sep beads is dependent on pH. If eluting DNA with water, make sure that the pH is around 7.5-8.0.

10. **Yield and quality of DNA**: Determine the absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

    DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/ml

    Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 55%-80%. The ratio of (absorbance$_{260}$)/(absorbance$_{280}$) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis.

Trouble Shooting Guide

Kit Contents

Product Number	D2510-00	D2510-01	D2510-02
Purification times*	10 Preps	150 Preps	500 Preps
Ultra-Sep® Binding Buffer	5 mL	2 x 60 mL	3X100 mL
Ultra-Sep® Beads	110 µL	1.7 mL	5.5 mL
DNA Wash Buffer	5 mL	2 x 20 mL	2 x 40 mL
Elution Buffer	5 mL	20 mL	60 mL
Instruction Booklet	1	1	1

Number of purification is based on the 100mg agarose gel. Routinely purification from 100mg gel which contains DNA fragments <100bp or gel are >2% agarose require additional Binding Buffer to extract full number of extractions. (Binding Buffer can be purchased separately, product # Binding-200).

Materials Supplied By User:

- Water bath equilibrated to 50~55°C
- Microcentrifuge capable of at least 10,000 x g.
- Nuclease-free 1.5 ml centrifuge tubes.
- Sterile deionized water (or TE buffer)
- Absolute (or 96-100%) ethanol.
- Protective eye-ware.
- 5M Sodium acetate, PH 5.2.

IMPORTANT	**DNA Wash Buffer** must be diluted with absolute ethanol (~96-100%)as follows: D2510-00 Dilute with 20 ml ~96-100% ethanol D2510-01 Dilute with 80 ml ~96-100 % ethanol D2510-02 Dilute with 160 ml ~96-100 % ethanol/bottle **Store diluted DNA Wash Buffer at room temperature.**

Introduction

Gel purification of DNA is a common technique for isolation of specific fragments from reaction mixtures. However, most methods either fail to completely remove agarose (which can lead to problems in downstream manipulations), shear the DNA, or result in very low yields.

The Ultra-Sep® Gel Extraction kit is one of the most economical and convenient kit available for the isolation of DNA fragments from agarose gel. By combining the silica particle method and Omega Bio-Tek's unique buffer system, this kit offers an easy and flexible protocol. The DNA band of interest is excised from gel, dissolved in Ultra-Sep® Binding Buffer, and then mixed with Ultra-Sep® Beads that bind DNA. Following a rapid wash step, DNA is eluted with deinoized water (or low salt buffer such as TE buffer) and is ready for other applications. The product is suitable for ligations, PCR sequencing, restriction digestion, or various labeling reactions.

Benefits

The Ultra-Sep® Gel Extraction Kit means:

- Speed - DNA recovery from agarose in <15 min
- Reliability - Optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - Purified DNA suitable for any application
- Flexibility- Less technique dependent

Storage and Stability

All Ultra-Sep® Gel Extraction Kit components are guaranteed for at least to 24 months from the date of purchase when stored at 22-25°C. Ensure that the bottle of Binding Buffer is capped tightly when not in use.

Binding Capacity

5 µl Ultra-Sep® Beads can bind up to 2 µg DNA.

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Ultra-Sep® Binding Buffer added to gel.	Volume of agarose gel slice determined incorrectly. Add enough Binding Buffer as instructed.
	Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 50°C to 55°C and allow gel to completely melt. Add more binding buffer if necessary.
	Inappropriate elution buffer	Check pH of the water or use 10mM Tris-HCl, pH 9.0 to elute DNA.
	TBE running buffer not fresh.	With overuse, TBE loses its buffering capacity and increases in pH. This raises the pH of the agarose/ DNA/ Binding Buffer solution which interferes with DNA binding to HiBind® matrix. Adjust pH by adding 5 ul of 5M sodium acetate pH 5.2 to the gel slice at the adsorption step. Use freshly prepared TBE buffer for gel purification (and prevent contamination of isolated DNA in addition to improving yields).
No DNA eluted	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate as instructed above.
	Incorrect amount of Binding Buffer added.	Add enough volume of Binding Buffer. For DNA fragments <200 bp add 3 x volume Binding Buffer.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A_{260}.	Make sure to wash pellet as instructed in steps 5 and 6. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
DNA sample floats out of well while loading agarose gel	Ethanol not completely removed following wash steps.	Dry the pellet as instructed in step 7 to dry before proceeding to elution step.

Ordering Information

Guidelines

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. Ultra-Sep® Gel Extraction Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

A. Solubilization of Agarose Gel

The Ultra-Sep® Binding Buffer is optimized to dissolve any type or grade of agarose gel. It does not contain NaI which is difficult to remove from DNA samples and cause low efficiency for downstream enzymatic reactions.

This standard Ultra-Sep® protocol is used to extract DNA fragments from 0.3%-2% agarose gel in TAE or TBE buffer. The volume of Ultra-Sep® Binding Buffer to add to a piece of agarose gel should be **approximately equal volume** or weight of the gel slice (e.g., 100 µl Ultra-Sep® Binding Buffer is added to 100mg gel slice) and incubated at 50°C for 7-10 minutes or until the gel slice is dissolved.

For DNA fragments less than 400 bp, the volume of Ultra-Sep® Binding Buffer to add to a piece of agarose gel should be **approximately 3 times** of volume or weight of the gel slice.

For > 2% agarose gels, the volume of Ultra-Sep® Binding Buffer to add to a piece of agarose gel should be **approximately 3 times of volume** or weight of the gel slice.

B. DNA Binding Efficiency

The DNA binding efficiency to the Ultra-Sep® beads is affected by the salt and pH of Ultra-Sep® Binding Buffer/gel mixture. DNA fragments less than 100bp can be more efficiently bound to the Ultra-Sep® beads at higher salt concentrations, while large DNA fragments are bound to Ultra-Sep® beads at lower salt concentrations.

Adsorbance of DNA to the Ultra-Sep® beads is also pH dependent. DNA will more efficiently bind to the Ultra-Sep® beads when the pH is less than 7.0. Ultra-Sep® Binding Buffer contains pH indicator. When the dissolved mixture of Ultra-Sep® Binding Buffer/gel is red in color, it means that the pH of the sample mixture exceeds 7.0. Adjust the pH by adding a small volume of Sodium Acetate (pH 5.2).

Ultra-Sep® Gel Extraction Kit Protocol

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. **It is strongly recommended, however, that fresh TAE buffer be used as running buffer.** *Do not reuse running buffer* as its pH will increase and reduce yields. TBE may also be used, but it has to be freshly prepared.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a UV light box, ensuring that as much agarose gel as possible has been removed. **Avoid more than 30 seconds exposure of UV light to the DNA**. *Always use protective eye wear when working with UV light.*

3. **Determine the approximate volume of the gel slice by weighing it in a clean 1.5 ml microfuge tube**. Assuming a density of 1 g/ml of gel, the volume of gel is derived as follows: a gel slice of mass 0.2 g will have a volume of 0.2 ml. **Add Ultra-Sep® Binding Buffer equal to gel volume. Resuspend Ultra-Sep® beads by vortexing and transfer 10 µl Ultra-Sep® beads to the sample. Incubate the mixture at 50°C-55°C for 10 min or until the gel has completely melted.** Mix by shaking or vortexing the tube every 2 minutes during the incubation. For DNA fragments less than 400bp or >2% agarose gels, use Binding Buffer equal to 3 times of volume or weight of the gel slice.

   Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel completely dissolves. DNA yield will significantly decreased when pH > 8.0. If the color of the mixture become orange or red, Add 5 µl of 5M sodium acetate, pH 5.2, to bring the pH down. After this adjustment, the color of the gel/Binding Buffer mixture should be light yellow.

4. Centrifuge in a microcentrifuge at 10,000 x g for 1 min at room temperature to pellet the beads. Discard the liquid.

5. **Wash the glass beads by adding 300 µl Ultra-Sep® Binding Buffer** and resuspend the pellet by vortexing. Centrifuge at 10,000 x g for 1 min. Discard the liquid.

   Note: The extra Binding buffer can be ordered separately, see catalog or call customer service for detail information.

6. **Add 750 µl of DNA Wash Buffer diluted with absolute ethanol** and resuspend the pellet by vortexing. Centrifuge at 10,000 x g for 1 min to pellet the beads.

   Note: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use.