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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 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) and 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

Binding Capacity

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

Kit Contents

Product Number	D2510-01	D2510-02
Purifications	150	300
Ultra-Sep™ Binding Buffer	60 mL	125mL
Ultra-Sep™ Beads	1.0mL	1.8mL
Wash Buffer Concentrate	100 mL	250 mL
Instruction Booklet	1	1

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.

Materials Supplied By User:

- Water bath equilibrated to 50-55°C
- Microcentrifuge capable of at least 10,000 x g.
- Sterile 1.5 ml centrifuge bottles.
- Sterile deionized water (or TE buffer)
- Absolute (or 95%) ethanol.
- Protective eye-ware.
- 5M Sodium acetate, PH 5.2.
- 1% Sobital solution

IMPORTANT	Wash Buffer Concentrate must be diluted with absolute ethanol as follows:	
	D2510-01	Dilute with 150 ml 100 % ethanol
	D2501-02	Dilute with 375 ml 100 % ethanol

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 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 3-4 times the volume of the volume or weight of gel slice (e.g., 300 µl Ultra-Sep™ Binding buffer is added to 100mg gel slice) and incubate 50-55°C for 10-15 minutes or until gel slice is dissolved. TBE gel usually cause lower yield.

B. DNA binding efficiency
The DNA binding efficiency to the Ultra-Sep™ beads is effected 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 the large DNA fragments are bound to Ultra-Sep™ beads at lower salt concentrations.

Adsorb of DNA to the Ultra-Sep™ beads are also pH dependent. DNA will be more efficient to 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 in red color, it means that the pH of sample mixture is exceeds 7.0. Adjust the pH by add smaller volume of Sodium Acetate (pH 5.2).

Ultra-Sep™ Gel Extraction 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 fresh 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-ware 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 3-6 X the gel volume. Normally, Incubate the mixture at 50°C-55°C for 10 min or until the gel has completely melted. Mix by shake or vortex the tube in every 2-3 minutes.

DNA fragments < 150 bp	6 volume of Ultra-Sep™ Binding buffer
DNA fragments > 4000 bp	3 volume of Ultra-Sep™ Binding buffer
> 2% agrose gel	6 volume of Ultra-Sep™ Binding buffer
Agarose gel in TBE buffer	5 volume of Ultra-Sep™ Binding buffer and 0.2 volume of 1% Sobital solution

Important: Monitor the pH of the Gel/Binding buffer mixture after the gel completely dissolved. 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. Apply the DNA/agarose solution to 10 µl Ultra-Sep™ beads and incubate at room temperature for 10 minutes. Centrifuge in a microcentrifuge at 10,000 x g for 1 min at room temperature to pellet the beads. Discard the liquid.

5 Optional: Wash the column by adding 300 µl Ultra-Sep™ Binding buffer and resuspend the pellet by votex. Centrifuge at 10,000 x g for 1 min. Discard the liquid.

6. Add 750 µl of Wash Buffer diluted with absolute ethanol and resuspend the pellet by votex for 3-5 minutes. Centrifuge at 10,000 x g for 1 min to pellet the beads.

7. Discard liquid and repeat step 6 with another 750 µl Wash Buffer.

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

9. Add 30-50 µl (depending on desired concentration of final product) sterile

deionized water (or TE buffer (pH 8.0) to the tube. Resuspend the pellet by votex. Incubate at 50-55°C in a water bath for 5 minutes. Centrifuge 1 min at 10,000 x g pellet the Ultra-Sep™ beads .

10 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 column is dependent on pH. If eluting DNA with water, make sure that the pH is around 7.5-8.0.

11 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:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor)\ \mu g/ml$$

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

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 50oC 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	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate as instructed above.
	Incorrect amount of Binding Buffer added.	Add 4.5-5 X volume of Binding Buffer. For DNA fragments <200 bp add 6 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.

Order Information on DNA/RNA Clean-up:

Product No.	Product Name	Description
D2501-01/02	Gel Extraction Kit (Q-spin column)	DNA purification from agarose gel by spin column format
D2500-01/02	Gel Extraction Kit (V-spin column)	DNA purification from agarose gel by spin column format. Vacuum/Spin protocol is also available.
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
D6537-01/02 D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Purification Kit	Isolate DNA from polyacrylamide gel
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
R6537-01/02 R6538-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format

* All OBI products available with size if 50 preps and 200 preps. Product number end with"-01" represent 50 preps kit and "-02" represent 200 preps kit.