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Introduction

While advances in oligonucleotide synthesis have allowed rapid, facile and efficient production of DNA fragments, purification can be time-consuming and occasionally ineffective. In some applications, there is no need for purified product. However, when purification is necessary, the two traditional techniques used for the purification of synthetic oligonucleotides or double stranded DNA fragments ≤ 100 bp are polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC). While HPLC remains a potent analytical tool, PAGE and its distant cousin capillary electrophoresis (CE) are probably most often used for the analysis of synthetic oligonucleotides. With UV shadowing (or ethidium bromide staining), PAGE allows the rapid purification of small quantities of synthetic DNA and is especially useful for parallel purification of several samples.

The E.Z.N.A.® Poly-Gel DNA Extraction Kit combines the simplicity of PAGE with the power of HiBind® technology to allow rapid and consistent recovery of ssDNA or dsDNA from acrylamide gels. Following PAGE, oligonucleotides are first visualized by UV shadowing or ethidium bromide staining and then eluted in 1-4 h using EDTA-containing buffer to eliminate degradation of DNA by contaminating DNase. A specially formulated buffer is then added to the eluate which is passed through a HiBind® DNA spin column. The bound DNA is finally washed to remove salts, buffers, and any protein contaminants, and then eluted with sterile deionized water or low salt buffer. Organic extractions and alcohol precipitations are eliminated reducing sample handling time and increasing yields. Furthermore, the spin column format allows parallel processing of multiple samples.The purified DNA is suitable for PCR, ligation reactions, hybridization techniques, or other procedures.

Storage and Stability

All components of the E.Z.N.A.® Poly-Gel DNA Extraction Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. Under cool ambient conditions (such as occurs during shipping) a precipitate may form in buffers. Simply warm to dissolve the precipitate.

Kit Contents

Product Number	D2561-00	D2561-01	D2561-02
HiBind® DNA columns	5	20	50
Poly-Gel Filter Units	5	20	50
2 ml collection tubes	10	40	100
Poly-Gel DNA Elution Buffer	5 ml	10 ml	20 ml
Buffer HB	10 ml	40 ml	90 ml
SPW Wash Buffer Concentrate*	2 ml	5 ml	10 ml
Instruction Manual	1	1	1

IMPORTANT	SPW Wash Buffer Concentrate must be diluted with absolute ethanol as follows:
	D2561-00 Add 8 ml absolute ethanol (96-100%)
	D2561-01 Add 20 ml absolute ethanol (96-100%)
	D2561-02 Add 40 ml absolute ethanol per bottle

Overview of Procedure

- Fractionate DNA using polyacrylamide gel.
- Visualize DNA fragment of interest and excise from gel.
- Crush/grind gel slice.
- Elute DNA with Poly-Gel DNA Elution Buffer.
- Purify eluate using HiBind® DNA column.

Before Starting

Prepare the following

- Water bath or incubator equilibrated to 65°C.
- For isotopically labeled DNA, autoradiographic film.
- Dilute SPW Wash Buffer Concentrate with absolute ethanol and store at room temperature.
- Microcentrifuge capable of 10,000 x g.
- Sterile 1.5 ml microcentrifuge tubes.

PAGE Methods

Acrylamide gels are useful for separation of small DNA fragments, typically oligonucleotides <100 base pairs. These gels are usually of a low acrylamide concentration (≤6%) and contain the non-ionic denaturing agent urea (7 M). For gel composition and buffers for your particular requirements, please refer to the Appendix.

Visualization of DNA

Fluorescence Shadowing

DNA fragments resolved on polyacrylamide gels can be visualized by the method of **UV shadowing**. This method is straight forward and may gives higher yields compared to staining methods (see DNA Staining below). In this technique the gel is placed on top of a flourescent material, usually a flourescent TLC silica plate. The gel is then illuminated by a UV light source. DNA bands in the gel will block transmittance of the UV light to the substrate.This will result in a dark area (i.e. non-fluorescing) area on the substrate

1. The gel must be removed from glass plates (glass blocks UV light and will prevent visualization by either UV shadowing or staining) and wrapped in plastic wrap to aid in handling and marking. Remove the top glass plate, and lay a sheet of plastic wrap over the gel, then flip the gel & glass plate over and carefully peel the gel away from the bottom glass plate. Wrap the gel entirely in the plastic wrap.

   TIP: Use only a single layer of plastic wrap and try to prevent air bubbles from forming between the gel and plastic wrap. These bubbles can scatter the UV light and make visualization difficult.

2. Place the gel on top of the dull white side of a fluor-coated TLC plate (Ambion, 10110 or Merck 60F254) and remove the plastic wrap on top of the gel. Hold a hand-held short-wavelength (254 nm) UV light source over the gel. (Long wavelength UV light will not work). The TLC plate beneath the gel should glow bright purple except wherever nucleic acids are present. A DNA band will appear as a dark shadow. The limit of sensitivity is about 0.3 µg in a single band.

   TIP: UV shadowing works for either DNA or RNA, labeled or unlabeled, so this technique has many other applications; *e.g.* for visualizing restriction enzyme digests.

DNA Staining

As an alternative to UV shadowing the acrylamide gel may be stained with acridine orange or ethidium bromide (EtBr) and held over a UV transilluminator to visualize the location of DNA bands within the gel.
If the DNA is to used as a probe, it is important that the stain be completely removed before hybridization as it will compromise hybridization efficiency. We recommend staining with acridine orange as opposed to EtBr since acridine orange will be removed from the probe by subsequent purification with HiBind® DNA spin columns. (EtBr can be used, but requires multiple butanol extractions to subsequently remove it before applying sample to spin column).

1. Remove gel from glass plates as described for UV shadowing.

2. Remove gel from the plastic wrap and place in a 2.0 µg/ml acridine orange solution for 15 minutes. Destain the gel in distilled water for 10 minutes. Then re-wrap the gel in plastic wrap for easier handling, and place the gel on a UV transilluminator to visualize DNA.

3. Carefully cut out (using a nuclease-free scalpel or razor blade) the smallest gel-fragment possible which contains the DNA band (corresponds to bright band in the gel). The smaller the size of this gel fragment, the better the elution efficiency (*i.e.* more probe will be recovered more quickly). If you are concerned that not all the probe was cut out, visualize the gel again with UV light to verify that the probe band is gone.

Isotopically Labeled DNA

If the DNA band of interest has been labeled with ^{32}P, ^{33}P, or ^{35}S for use as a probe, it can be readily visualized by autoradiography.

1. Following PAGE separate the glass plates, leaving the gel adhered to the larger glass plate. Wrap a piece of plastic wrap over the gel. If the glass and gel will not fit into the film cartridge, then both glass plates should be carefully removed and the gel wrapped entirely in plastic wrap (for easier handling).The gel is ready to expose to film.

2. Place the gel (sandwiched between the glass and plastic wrap) against the film so that the film is closest to the gel. The film can simply be aligned with one corner of the glass plate, the corners and sides of the glass plate marked directly on the film with a permanent marker, or alternatively, radioactive ink can be used for orientation. One corner of the film (*e.g.* bottom right corner) is usually snipped or folded up so that the glass and gel can be aligned with the film after developing.

3. Expose the gel to autoradiographic film, about 30 seconds for a high specific activity ^{32}P-labeled probe and 10 minutes for a low specific activity ^{32}P-labeled probe or high specific activity ^{35}S-labeled probe. The aim is to get an exposure of a light gray band so that a thin gel fragment can be excised from the gel. Realign the glass plate and gel with the developed film (using the guide marks made earlier) and carefully excise the band using a nuclease-free scalpel or razor blade.The smaller the size of this gel fragment, the better the elution efficiency (*i.e.* more probe will be recovered more quickly). The gel can be re-exposed to insure that the gel and film were properly aligned and that the probe was excised.

   TIP: If possible, run markers or a known size standard so that the appropriate band is selected. If no markers have been run, the bromophenol (dark blue) and xylene cyanol (light blue) dyes can serve as size references. See Appendix for dye migration under different conditions.

Poly-Gel DNA Extraction Procedure

Please take a few minutes to read and familiarize yourself with this procedure. Make sure all necessary reagents and equipment are ready before starting. Wash Buffer Concentrate must be diluted with absolute ethanol as indicated in **Before Starting** on page 3 and stored at room temperature.

1. Transfer the gel fragment onto a nuclease-free microscope slide. With a second glass slide (or nuclease-free razor) mash and pulp the gel completely. Carefully transfer gel pulp to a nuclease-free microcentrifuge tube and add 250 µl Poly-Gel DNA Elution Buffer. This volume is usually enough to submerge a slice 2 mm x 10 mm x 0.8 mm. For a larger fragment adjust volume of Poly-Gel DNA Elution Buffer used until the gel is covered. Any buffer or dH_2O can be used; however, we recommend the Poly-Gel DNA Elution Buffer supplied in order to prevent DNA degradation by exogenous nucleases.

2. Incubate the gel fragment in Poly-Gel DNA Elution Buffer 1-4 h at 65°C.The elution time is dependent on the size of the gel fragment, DNA size and the temperature of the incubation. We find that about 70% of a 100 bp DNA fragment elutes in approximately 4 hrs at 65°C. Larger fragments will take longer to elute.

3. Transfer gel and buffer to a Poly-Gel filter unit mounted in a 2 ml collection tube. Use a blue pipette tip with the end cut to do this. Centrifuge at 10,000 x g for 10 minutes at room temperature to filter the eluted DNA.

   Note: If isolating labeled DNA probes for use in hybridization assays, no further purification is necessary. An aliquot of the eluted probe can be used directly in the hybridization reaction. *An optional phenol: chloroform extraction may be performed. However do not extract with phenol if the DNA probe is labeled with digoxygenin as DNA will separate into the organic phase.* Also a standard etahanol precipitation with carrier (glycogen, tRNA, or linear acrylamide) may be performed for further clean-up.

DNA Clean Up with HiBind® Columns

Proceed to step 4 only if downstream applications require enzymatic manipulation of DNA, such as with PCR. Use the eluted material from step 3 directly.

4. To the eluate from step 3 add 5 x vol Buffer HB and vortex briefly to mix. For fragments smaller than 100 bp, use at least 6 x vol Buffer HB. In such cases, adding 5-10 µg yeast tRNA as carrier will also increase the yield of DNA recovered. The volume of carrier tRNA should be no more than 1/10 x volume Buffer HB added.

5. Apply 750 µl of the mixture to a clean HiBind® DNA column mounted in a 2 ml collection tube (provided). Centrifuge at 10,000 x g for 1 min at room temperature. Discard the flow-through and reuse the collection tube in step 6.

6. Add the remaining mixture to the HiBind® column and centrifuge as above. Discard flow-through and place column back into 2 ml collection tube.

7. Wash the column by adding 700 µl SPW Wash Buffer. Centrifuge at 10,000 x g for 1 min at room temperature.

   NOTE: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. Follow directions on bottle. DNA Wash Buffer should be at room temperature to ensure effective washing.

8. Discard flow-through and using the same collection tube centrifuge 2 min at 10,000 x g to dry the column.

   NOTE: This step is critical for effectively removing traces of ethanol from the column.

9. Transfer column to a sterile 1.5 ml microfuge tube and add 15-30 µl sterile dH_2O (or low salt buffer) directly onto HiBind® matrix. Centrifuge at 10,000 x g for 1 minute to elute bound DNA. For DNA <100 bp approximately 80% will be recovered with a single elution. Subsequent elution steps each yield 80% of the remaining bound material. For expected yields greater than 20 µg, repeat the elution with another 15-30 µl water.

Quantification of DNA by UV absorption at 260 nm

Oligonucleotides are often quanatitated in A_{260} units. Although controversial, the practical definition is that 1 A_{260} is the amount of oligonucleotide, which dissolved in 1.0 ml buffer, measured in a 1.0 cm path length cuvette, at 260 nm gives absorbance 1.0.

1. Dilute an appropriate amount of purified DNA in dH_20 or TE buffer.The sample should give an absorbance value between 0.1 and 0.5. However if expected yields $\leq$ 5 µg then observed OD values will be lower.

2. Measure the absorbance in a clean 1-cm quartz cell at 260 nm.

3. Calculate the extinction coefficient (ε) of the product by the addition of the individual extinction coefficients of every nucleotide not taking into account hypochromicity effects. The epsilon-values are:

Nucleoside	ε
dT	8.8 cm^2/µmol
dC	7.3 cm^2/µmol
dG	11.7 cm^2/µmol
dA	15.4 cm^2/µmol

4. Calculate the sample concentration with Lambert-Beer law:

$$A= [\varepsilon]_{oligo} \times C \times l$$

where A= absorbance at 260 nm, $[\varepsilon]_{oligo}$= is the sum of the individual extinction coefficient for the nucleosides, C the µmolar concentration, and l= the path of the quartz cell (1 cm). Remember that a solution 1 µM has 1 pmol/µl of solute. Also for dsDNA $[\varepsilon]_{oligo}$ should be doubled.

Appendix

Trouble Shooting Guide

Problem	Possible Cause	Suggestion
Little or no DNA recovered	Too little Buffer HB added to eluate.	Measure volume of eluate and add 4.5 to 5 x volumes of Buffer HB. For DNA <100 bp it may be necessary to add more.
	DNA band not excised from gel.	Inspect gel to ensure band is removed.
	Incomplete elution from acrylamide gel.	Increase incubation time with Poly-Gel DNA Elution Buffer.
	No ethanol added to Wash Buffer Concentrate.	Add absolute ethanol to Wash Buffer prior to use.
	Starting material too small.	Increase starting material or assess yield by performance in downstream application.
Poly-Gel filter unit clogged	Acrylamide gel not crushed before adding Poly-Gel DNA Elution Buffer.	Completely mince gel fragment as indicated using razor blade or microscope slide method.

Acrylamide Gels for DNA

Prepare the acrylamide-bisacrylamide (29:1) gel with 7 M urea 100 mM Tris-borate and 2 mM EDTA.

Table 1. Denaturing PAGE for oligonucleotide separation

Final AA concentration	8%	12%	20%
40% acrylamide-bisacrylamide (29:1)	12 ml	18 ml	30 ml
Urea	30 g	30 g	30 g
Distilled water	18.5 ml	12.5 ml	0.5 ml
1.0 M Tris-borate (pH 8.3), 20 mM EDTA	6 ml	6 ml	6 ml
10% ammonium persulfate	420 µl	420 µl	420 µl
TEMED	20 µl	20 µl	20 µl

Mix all the ingredients, except the ammonium persulfate and TEMED in a round bottle and evacuate to degas. Add ammonium persulfate and TEMED just prior casting the gel. 60 ml are enough for a 400 x 200 x 0.5 mm gel.

Running buffer: 100 mM Tris-Borate pH 8.3, 2 mM EDTA.

Choose the proper gel concentration from the table bellow.

Table 2: Mobility of DNA during gel electrophoresis

Gel concentration	Separation range	Bp corresponding to bromophenol blue	Bp corresponding to xylene cyanol
Polyacrylamide (30:1) - ds linear DNA			
3.5%	25-500	100	450
5.0%	15-300	60	270
8.0%	5-200	25	150
Urea-polyacrylamide (19:1) - ss linear DNA			
5.0%		30	125
6.0%		25	110
8.0%		20	75
20.0%		7	27

Ordering Information

Product	Product #
E.Z.N.A.® Poly-Gel DNA Extraction Kit For recovery of DNA from polyacrylamide gel slices, 20 or 50 isolations	D2561
E.Z.N.A.® Poly-Gel RNA Extraction Kit For recovery of RNA from polyacrylamide gel slices, 20 or 50 isolations	R6376