**EZgeneTM Poly-Gel DNA Purification Kit**

**(BW-DC3512)**

Contents

[Kit Contents 1](#_Toc133225806)

[Introduction 2](#_Toc133225807)

[Storage and Stability 2](#_Toc133225808)

[Safety Information 2](#_Toc133225809)

[Before Starting 3](#_Toc133225810)

[PAGE Method 3](#_Toc133225811)

[Visualization of DNA 3](#_Toc133225812)

[DNA Staining 4](#_Toc133225813)

[Isotopically Labeled DNA 5](#_Toc133225814)

[Poly-Gel DNA Extraction Procedure 6](#_Toc133225815)

[DNA Clean Up with ezBind Columns 7](#_Toc133225816)

[Quantification of DNA by UV absorption at 260 nm 8](#_Toc133225817)

[Acrylamide Gels for DNA 9](#_Toc133225818)

[Trouble Shooting Guide 10](#_Toc133225819)

[Limited Use and Warranty 11](#_Toc133225820)

# Kit Contents

|  |  |  |  |
| --- | --- | --- | --- |
| **Product** | **BW-DC3512-00** | **BW-DC3512-01** | **BW-DC3512-02** |
| Preps | 4 | 50 | 250 |
| Mini Columns | 4 | 50 | 250 |
| 2.0 mL Collection Tubes | 4 | 50 | 250 |
| 1.5 mL Microfuge Tubes | 4 | 50 | 250 |
| Poly-Gel Filter Units | 4 | 50 | 250 |
| Gel Elution Buffer | 1.2 mL | 15 mL | 70 mL |
| Buffer GC | 8 mL | 100 mL | 2×250 mL |
| DNA Wash Buffer | 2 mL | 15 mL | 3×24 mL |
| Elution Buffer | 500 μL | 7 mL | 30 mL |
| User Menu | 1 | 1 | 1 |

# Introduction

The EZgeneTM Poly-Gel DNA Extraction Kit is designed for 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 cut out, soaked and eluted from using gel elution buffer. A specially formulated binding buffer is then added to the eluate to allow the binding of the DNA to the silica matrix. While proteins, salts, buffers, and other impurities are removed by wash buffer, the DNA is eluted sterile deionized water or low salt buffer. In addition, 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 EZgeneTM Poly-Gel DNA Extraction Kit are stable for at least 12 months from date of production when stored at room temperature(15-25℃). Under cool ambient conditions (such as occurs during shipping) a precipitate may form in buffers, simply warm up the solution to dissolve the precipitates.

# Safety Information

Buffer GC contains chaotropic salts that are irritants. Do not add bleach directly to the experiment waste. Take appropriate laboratory safety measures and wear gloves when handling.

# Before Starting

* Water bath or incubator equilibrated to 65°C.
* For isotopically labeled DNA, autoradiographic film
* Microcentrifuge capable of 10,000 x g
* Sterile 1.5 mL microcentrifuge tubes.
* Dilute DNA Wash Buffer with absolute ethanol as follows
	+ **BW-DC3512-00** Add 8 mL absolute ethanol
	+ **BW-DC3512-01** Add 60 mL absolute ethanol
	+ **BW-DC3512-02** Add 96 mL absolute ethanol

# PAGE Method

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.

* + 1. Place the gel on top of the dull white side of a fluor-coated TLC plate,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 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 ezBind 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 32P, 33P, or 35S 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 32P-labeled probe and 10 minutes for a low specific activity P-labeled probe or high specific activity 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.

**Note**: 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

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 Gel 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 Elution Buffer used until the gel is covered. Any buffer or ddH2O can be used; however, we recommend the Elution Buffer supplied in order to prevent DNA degradation by exogenous nucleases.
2. Incubate the gel fragment in Elution Buffer 1-4 hrs at 65℃.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℃. Larger fragments will take longer to elute.

1. Transfer Gel and Buffer to a **White Poly-Gel Filter Unit** mounted in a sterile

1.5 mL microcentrifuge tube. Use a blue pipette tip with the end cut to do this. Centrifuge at 10,000 x g for 10 min 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 ethanol precipitation with carrier (glycogen, tRNA, or linear acrylamide) may be performed for further clean-up.

## DNA Clean Up with ezBind 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.

1. To the eluate from step 3 add ***5 volumes Buffer GC*** and vortex briefly to mix. For fragments smaller than 100 bp, use at least 6 x Vol Buffer GC. In such cases, adding 5-10 pg 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 GC*** added.
2. nsert a Mini column into a 2.0 mL collection tube.Apply ***700 μL*** of ***the mixture*** to the Mini column. Centrifuge at 10,000 x g for 1 min at room temperature. Discard the flow-through and reuse the collection tube in step 6.
3. Add the remaining mixture to the Mini column and centrifuge as above. Discard flow-through and place Mini column back into 2.0 mL collection tube.
4. Wash the Mini column by adding ***650 μL DNA Wash Buffer***. Centrifuge at 10,000 x g for 1 min at room temperature, Discard flow-through. ***Repeat once.***

**NOTE**: DNA 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.

1. Discard flow-through and centrifuge the empty Mini column, ***with the lid open***, at top speed for 2 min.

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

1. Transfer Mini column to a sterile 1.5 mL microfuge tube and add ***50 μL Elution Buffer or sterile ddH2O (or low salt buffer)*** directly onto ezBind matrix. Centrifuge at 10,000 x g for 1 min to elute bound DNA. For DNA<100 bp approximately 80% will be recovered with a single elution. Reload the eluate into the Mini Column and centrifuge again to improve the recovery.

# Quantification of DNA by UV absorption at 260 nm

Oligonucleotides are often quanatitated in A260 units. Although controversial, the practical definition is that 1 A260 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 ddH2O or TE buffer. The sample should give an absorbance value between 0.1 and 0.5. However if expected yields ≤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 (E) 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** | **E** |
| dT | 8.8 cm2 /pmol |
| dC | 7.3 cm2 /pmol |
| dG | 11.7 cm2 /pmol |
| dA | 15.4 cm2 /pmol |

* 1. Calculate the sample concentration with Lambert-Beer law:

A= [E] oligo x C x l

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

# Acrylamide Gels for DNA

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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 | 12.5mL | 0.5mL |
| 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:

|  |  |  |  |
| --- | --- | --- | --- |
| **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 |

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problem** | **Possible Cause** | **Suggestion** |
| **Little or no DNA recovered** | Too little Buffer GC added to eluate. | Measure volume of eluate and add4.5 to 5 x volumes of Buffer GC. 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 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 Elution Buffer. | Completely mince gel fragment as indicated using razor blade or microscope slide method. |

# Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO’s literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO’s sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.



**[Contact Us](https://www.sigmaaldrich.cn/CN/en/collections/offices):** 400-115-2855

[www.beiwobiomedical.com](http://www.beiwobiomedical.com)

**[Customer Support](https://www.sigmaaldrich.cn/CN/en/support/customer-support):**

market@beiwobiomedical.com

**Technical Support:**

tech@beiwobiomedical.com