TECHNICAL MANUAL

# DNA Purification Using the Gel and PCR Clean-up Kit

## Table of Contents

# 1.0 Introduction

The Gel and PCR Clean-up Kit uses a membrane-based system to quickly purify DNA fragments of 100 bp to 10 kb in length from excised standard or low-melt agarose gel fragments. It can also be used to purify products from enzymatic reactions such as restriction digestion or polymerase chain reaction (PCR) amplification, for efficient removal of enzymes, nucleotides, salts, and other impurities. The purified DNA is suitable for applications such as automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion, and in vitro transcription. Up to 40 µg of DNA can be processed per column in as little as 20 minutes and eluted in a volume as low as 15 µL, with up to 95% recovery depending on DNA fragment length. The Gel and PCR Clean-up Kit can be used to purify linear DNA fragments, supercoiled plasmid DNA, or single-stranded DNA (circular or linear). Expected yields with single-stranded DNA are lower than with double-stranded DNA.

The Gel and PCR Clean-up Kit is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. Band(s) of interest are excised following gel electrophoresis and dissolved in guanidine isothiocyante (Membrane Binding Solution). Alternatively, Membrane Binding Solution can be added directly to PCR amplification products. The DNA is then isolated by microcentrifugation, which forces the dissolved gel slice or enzymatic reaction product through the membrane while the DNA binds to the silica on the surface of the membrane. Ethanol-based washing then removes the salts, and DNA is eluted in nuclease-free water.

# 2.0 Materials, Reagents, and Equipment

## 2.1 Gel and PCR Clean-up Kit (Catalog #79030)

The components listed below are available as part of the Gel and PCR Clean-up Kit and are not available for individual sale. Each kit contains sufficient reagents for 250 purifications.

Refer to the Product Information Sheet (PIS) for the Gel and PCR Clean-up Kit (Document #10000005436) for component storage and stability information; the PIS is also available at www.stemcell.com, or contact us to request a copy.

| COMPONENT NAME | COMPONENT # | SIZE |
|---|---|---|
| DNA Minicolumns | 79031 | 250 Columns |
| Collection Tubes | 79032 | 250 Tubes |
| Membrane Wash Solution | 79033 | 75 mL |
| Membrane Binding Solution* | 79034 | 100 mL |
| Nuclease-Free Water | 79035 | 13 mL |

*Please refer to the Safety Data Sheet (SDS) for hazard information.

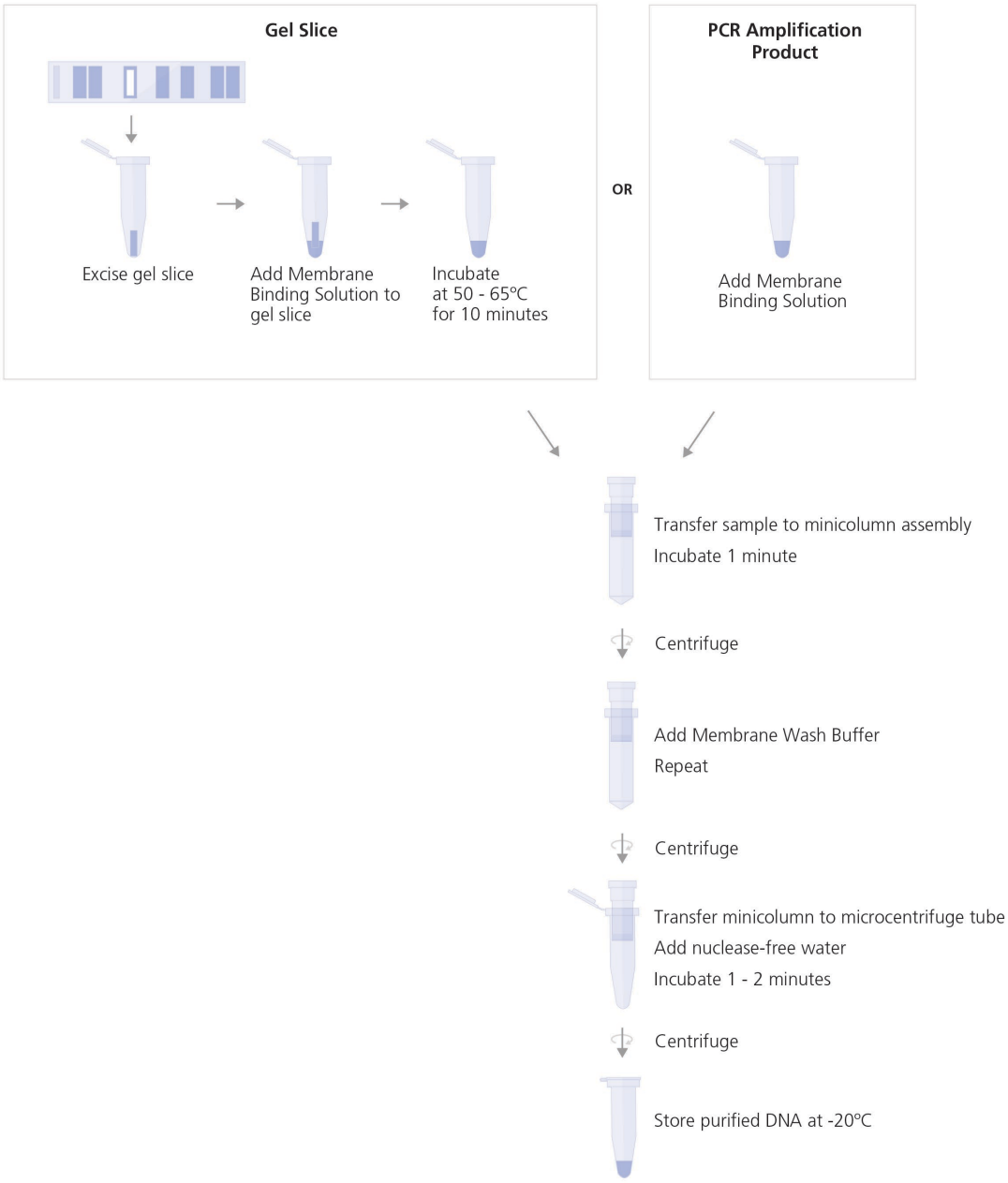
## 2.2 Additional Required Materials and Reagents

| PRODUCT | CATALOG # |
|---|---|
| 95% Ethanol, RNase-free | --- |
| Microcentrifuge tubes, 1.7 mL | e.g. 38089 |
| **For gel purification:** | |
| 50 - 65°C heating block | --- |

## 2.3 Equipment

- Biosafety cabinet certified for Level II handling of biological materials
- Microcentrifuge
- Pipettor and sterile tips
- -20°C freezer
- Refrigerator (2 - 8°C)

# 3.0 Protocol Diagram



# 4.0 Preparation of Membrane Wash Buffer

Prepare the Membrane Wash Buffer as follows:

1. Add 375 mL of 95% ethanol to 75 mL of Membrane Wash Solution. Mix thoroughly.
2. Label the bottle to indicate that ethanol has been added.
3. Store Membrane Wash Buffer at 15 - 25°C, tightly capped.

# 5.0 Sample Preparation

Prepare a gel slice (section 5.1) or PCR amplification product (section 5.2) before proceeding to DNA purification (section 6.0).

## 5.1 Gel Slice

DNA can be extracted from standard or low-melt agarose gels run with either TAE or TBE buffer.

1. Weigh a 1.7 mL microcentrifuge tube for each DNA fragment to be isolated. Record the weight.
2. Visualize and photograph the DNA using a long-wavelength UV lamp and an intercalating dye such as ethidium bromide.

   *Note: Irradiate the gel for the minimum time possible to reduce nicking.*
3. Using a scalpel or razor blade, excise the DNA fragment of interest in a minimal volume of agarose.
4. Transfer the gel slice to the weighed microcentrifuge tube and record the weight. Subtract the weight of the empty tube from the total weight to obtain the weight of the gel slice. Alternatively, tare (or zero) the scale with the empty tube prior to weighing the gel slice.

   *Note: The maximum capacity of the DNA Minicolumn is 350 mg of agarose gel mass dissolved in 350 µL of Membrane Binding Solution per column pass. For gel slices > 350 mg, continue to pass additional sample through the DNA Minicolumn until all of the sample has been processed. The maximum amount of agarose that can be processed through a single column is approximately 3.5 g (10 x 350 mg).*

   *Note: If not used immediately, store the gel slice at 2 - 8°C or at -20°C for up to 1 week in a tightly closed tube under nuclease-free conditions.*
5. Add Membrane Binding Solution at a ratio of 10 µL of solution per 10 mg of gel slice.
6. Mix the gel slice solution as follows:
   - For DNA fragments > 5 kb, mix gently by inversion
   - For DNA fragments < 5 kb, vortex to mix
7. Incubate the mixture at 50 - 65°C for 10 minutes or until the gel slice is completely dissolved. During incubation, mix the tube every few minutes as indicated in step 6 to increase the rate of dissolution.

   *Note: Gel slices with higher agarose concentrations (2 - 3%) may take longer to dissolve than 1% agarose gels, and may result in lower yields.*
8. Briefly centrifuge the tube at room temperature to ensure the contents are at the bottom of the tube.
9. Proceed to DNA purification (section 6.0).

## 5.2 PCR Amplification Product

Amplify the target of choice using standard amplification conditions. For amplification reactions that do not produce a single product, or where amplification has been inefficient and there is highly visible primer dimer, gel purification of the band of interest is recommended. Alternatively, use an 80% ethanol wash solution as a substitute for the Membrane Binding Solution in step 1 below and in the DNA purification protocol (section 6.0). This will reduce primer-dimer carryover.

1. Add an equal volume of Membrane Binding Solution to the sample.

   *Note: Mineral oil will not interfere with purification.*

2. Proceed to DNA purification (section 6.0).

## 6.0 DNA Purification

1. Place one DNA Minicolumn in a Collection Tube for each dissolved gel slice (section 5.1) or PCR amplification product (section 5.2).
2. Transfer the sample to the minicolumn assembly.

   *Note: The maximum capacity of the minicolumn is as follows:*

   - **For gel slices**: 350 mg of gel dissolved in 350 µL of Membrane Binding Solution per column pass. For gel slices > 350 mg and up to 3.5 g, repeat steps 3 - 5 until all of the sample has been processed.
   - **For PCR products**: 350 µL per column pass (PCR product + Membrane Binding Solution). For volumes > 350 µL and up to 2 mL total (1 mL PCR product + 1 mL Membrane Binding Solution), repeat steps 3 - 5 until all of the sample has been processed.
3. Incubate at room temperature (15 - 25°C) for 1 minute.
4. Centrifuge the minicolumn assembly at 16,000 x *g* for 1 minute.
5. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Place the minicolumn back in the Collection Tube.
6. Add 700 µL of Membrane Wash Buffer to the minicolumn. Centrifuge the minicolumn assembly at 16,000 x *g* for 1 minute.
7. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Place the minicolumn back in the Collection Tube.
8. Add 500 µL of Membrane Wash Buffer to the minicolumn. Centrifuge the minicolumn assembly at 16,000 x *g* for 5 minutes.
9. Remove the minicolumn assembly from the microcentrifuge, being careful not to wet the bottom of the column with the flowthrough. Remove the minicolumn from the Collection Tube, and discard the liquid in the tube. Place the minicolumn back in the Collection Tube.
10. Centrifuge the minicolumn assembly at 16,000 x *g* for 1 minute to dry membrane.
11. Carefully transfer the minicolumn to a clean DNase-free 1.7 mL microcentrifuge tube. Apply 50 µL of nuclease-free water directly to the center of the column, without touching the membrane with the pipette tip.

    *Note: For more concentrated DNA, an elution volume as low as 15 µL of nuclease-free water may be used. If using 15 µL, ensure the membrane is completely covered with nuclease-free water before centrifuging.*
12. Incubate at room temperature (15 - 25°C) for 1 - 2 minutes.
13. Centrifuge the minicolumn assembly at 16,000 x *g* for 1 minute. Discard the minicolumn. Store the microcentrifuge tube containing eluted DNA at 2 - 8°C or -20°C.

    *Note: The volume of eluted DNA will be approximately 42 - 47 µL. If the DNA needs to be further concentrated, perform an ethanol precipitation.*

# 7.0 Troubleshooting

| PROBLEM | POSSIBLE CAUSE/SOLUTION |
|---|---|
| Low DNA yield | Verify that an equal volume of Membrane Binding Solution was added to the gel slice or PCR product. |
| | Ensure the gel slice is completely dissolved before proceeding with purification. |
| | If the amount of purified DNA is too small to quantitate by spectrophotometry, quantitate using alternative methods such as fluorescence-based detection or by agarose gel electrophoresis followed by ethidium bromide staining. |
| | Ensure centrifuge speed is 16,000 x *g.* |
| | Verify that ethanol was added to the Membrane Wash Solution (section 4.0). |
| Poor results with automated fluorescent sequencing | Too little DNA may have been used. Increase the amount of DNA used in sequencing reactions, or concentrate the DNA by ethanol precipitation. Up to 7 µL of eluted DNA can be used per fluorescent sequencing reaction. |
| | Too much DNA can interfere with fluorescent sequencing. Use less eluted DNA or dilute DNA prior to sequencing. |
| | If TE was used for elution, ethanol-precipitate the DNA or repurify the DNA fragments and elute with nuclease-free water. |
| | Excessive thymidine-dimer formation may have occurred during UV exposure. Minimize exposure of gel to UV light. |
| Poor restriction digestion | Increase the amount of restriction enzyme and/or the length of incubation time. Digest at the appropriate temperature and in the optimal buffer for the restriction enzyme used. |
| | Ethanol or salt carryover into the eluted DNA may have occurred. Ethanol-precipitate the DNA or keep the DNA volume to ≤ 10% of the final reaction volume. |
| DNA yields on gel look low compared to spectrophotometric readings | Trace contaminants in eluted DNA can artificially inflate spectrophotometer readings. Use agarose gel electrophoresis followed by ethidium bromide staining to determine DNA yields. |
| | Ethanol-precipitate the DNA. |
| Low $A_{260}/A_{230}$ ratios | Typically due to guanidine isothiocyanate contamination. Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. If low $A_{260}/A_{230}$ ratio is a concern, ethanol-precipitate the DNA. |
| Clogged spin basket | To ensure the gel slice is completely dissolved, increase the incubation time at 50 - 65°C. |
| | Verify that an equal ratio of Membrane Binding Solution to gel slice mass is used. |
| Purified DNA floats out of the well when loaded on a gel | Ethanol carryover can cause this. Ensure that the Membrane Wash Buffer is not carried over from the wash steps. Empty the Collection Tube and recentrifuge the minicolumn assembly for 5 minutes to remove residual Membrane Wash Buffer. After washing, centrifuge the minicolumn assembly (section 6.0 step 10) to allow evaporation of any residual ethanol. |
| | Add DNA loading dye (e.g. Catalog #79018) to the DNA sample before loading onto the gel. |

| PROBLEM | POSSIBLE CAUSE/SOLUTION |
|---|---|
| Purified DNA bands are not sharp | DNA may be sheared. Mix the gel slice gently with Membrane Binding Solution. |
| | Nuclease contamination may be an issue. Autoclave the gel running buffer before use. |
| | Store the gel slice at 2 - 8°C or at -20°C for up to 1 week under nuclease-free conditions. |
| Low cloning efficiency | May be due to guanidine isothiocyanate contamination. Ethanol-precipitate the DNA, washing the pellet with 70% ethanol to reduce contamination. |

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