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# DNA Gel Extraction Kit

# Product Insert

**Product # 13100**

The Norgen DNA Gel Extraction Kit is designed for the rapid preparation and purification of DNA fragments that have been fractionated on agarose gels. The recovered DNA is free from agarose and other impurities, and is compatible with restriction enzyme digestion, ligation into vectors and sequencing.

## Norgen's Purification Technology

Purification is based on spin column chromatography. Norgen's column binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The DNA of interest is first run on an agarose gel. The DNA band is then excised from the gel using a razor blade, and the gel slice is transferred to a microcentrifuge tube (please see flow chart on page 3). Next, 3 volumes of Binding Buffer G are added to the gel slice and the tube is incubated at 55°C for up to 10 minutes. The Binding Buffer G contains chaotropic salts, which are used to both melt the agarose gel slice and also to allow reversible binding of the target DNA to the matrix. The sample containing the DNA and the melted agarose is then applied to one of the provided spin columns through centrifugation. Norgen's column binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while the agarose and other impurities will be removed in the flowthrough. The bound DNA is then washed twice using the provided Wash Solution A in order to remove any remaining impurities, and the purified PCR product is eluted with the Elution Buffer B.

## Specifications

| Kit Specifications | |
|---|---|
| Column Binding Capacity | 10 μg |
| Maximum Weight of Gel Slice | 400 mg |
| Average DNA Recovery | 70 – 90% |
| Size of DNA Purified | 100 – 15,000 bp |
| Minimum Elution Volume | 30 μL |
| Time to Complete 10 Purifications | 30 minutes |

## Advantages

- Fast and easy processing using a rapid spin-column format
- High recovery; purification of DNA fragments is up to 90% for inputs > 1 μg
- Compatible with agarose gel concentrations of up to 4%

## Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

**Kit Components**

| Component | Product # 13100 (50 samples) |
|---|---|
| Binding Buffer G | 80 mL |
| Wash Solution A | 12 mL |
| Elution Buffer B | 8 mL |
| Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

**Precautions and Disclaimers**
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*. Protective eyewear should be worn when working with UV light.

**Customer-Supplied Reagents and Equipment**

- Benchtop microcentrifuge
- 96 - 100% ethanol
- Isopropanol

# Procedure

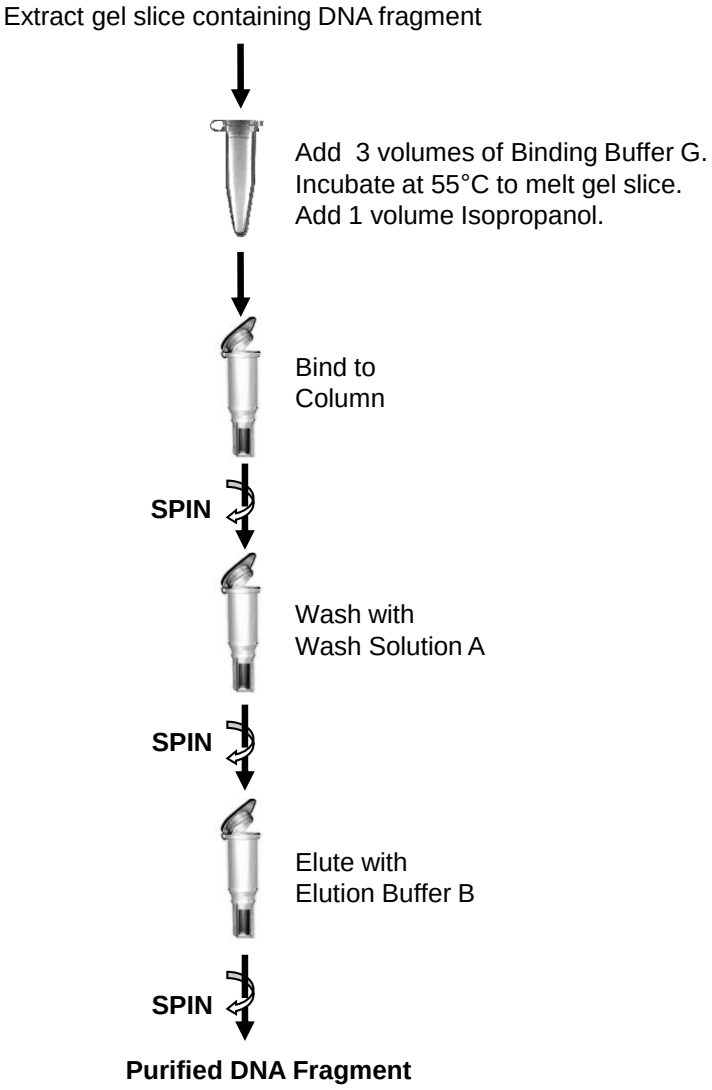
All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. Please check your microcentrifuge specifications to ensure proper speed. The correct RPM can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force. All centrifugation steps are performed at room temperature. Centrifugation at 4°C will not adversely affect kit performance

## Flow Chart

Procedure for the Rapid Purification of DNA Fragments Fractionated on Agarose Gels




**Notes prior to use:**

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use, and that no precipitation has occurred. If precipitation is observed, then the solutions should be warmed and mixed gently.
- Prepare a working concentration of **Wash Solution A** by adding 48 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated **Wash Solution A**. This will give a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.

1. **Excising DNA From Gel**
   - **a.** Run DNA fragment of interest on agarose gel.

     **Note:** It is recommended that fresh buffer be used for running the gel. A used one may have its buffering capacity exhausted and may subsequently reduce yields.

   - **b**. Excise fragment from gel using a scalpel or razor blade. Remove as much excess agarose as possible. Minimize exposure of DNA to UV light.
   - **c.** Place the excised agarose into a sterile and pre-weighed 1.5 mL microcentrifuge tube.

2. **Sample Preparation**
   - **a.** Determine the weight of the gel slice.
   - **b.** Add 3 volumes of the **Binding Buffer G** to 1 volume of gel (assuming that the gel has the same density as water so that 100 mg of gel occupies the same volume as 100 μL of **Binding Buffer G**). For example, add 300 μL of **Binding Buffer G** to a 100 mg gel slice.

     **Note:** For gels made with greater than 2% agarose, add 6 volumes of **Binding Buffer G**. For larger gel slices (greater than 300 mg) cut gel into smaller pieces to facilitate melting.

   - **c.** Incubate at 55°C for up to 10 minutes, or until completely dissolved. (Please see Appendix 2 for a time guideline). Vortex every 2 to 3 minutes to assist in dissolving. It is important to dissolve gel slice completely.
   - **d.** Add 1 volume of Isopropanol (supplied by user) to 1 volume of gel and mix. For example, add 100 μL of Isopropanol to a 100 mg gel slice.

     **Note:** For gels made with greater than 2% agarose, add 2 volumes of Isopropanol.

3. **Binding DNA to Column**
   - **a.** Assemble a column with one of the provided collection tubes. Apply up to 750 μL of sample to the column and centrifuge for 1 minute at 8,000 x g (~8,000 RPM). The maximum volume that the reservoir can accommodate during each spin is 750 μL. If a sample volume exceeds this, repeat spin as necessary until the entire sample has been processed.

     **Note:** Make sure that the sample has passed through the column. Spin an additional minute if required.

   - **b.** Discard the flowthrough, and reassemble the spin column and its collection tube.

4. **Washing Bound DNA**
   - **a.** Apply 500 μL of **Wash Solution A** to column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
   - **b.** Discard the flowthrough and reassemble the spin column with its collection tube.
   - **c.** Spin the column for 2 minutes at 14,000 x g (~14,000 RPM) in order to thoroughly dry the column. Discard the collection tube.

5. **Elution of Clean DNA**
   - **a.** Assemble the column with one of the provided 1.7 mL **Elution tubes**.
   - **b.** Add 30 – 50 μL of **Elution Buffer B** to the center of the column bed.

     **Note:** For more concentrated DNA, use 30 μL of **Elution Buffer B**. For slightly higher recoveries, use 50 μL.

   - **c.** Let stand at room temperature for 1 minute**.**
   - **d.** Centrifuge for 2 minutes at 14,000 x g (~14,000 RPM).

## Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | pH of the electrophoresis buffer was too high | Ensure that fresh running buffer is used for electrophoresis. When the buffer is re-used, it often exhibits increased pH and may subsequently reduce yields. |
| | Gel slice was not completely melted in the **Binding Buffer G** | The gel slice should be incubated at 55°C until completely dissolved. The slice should be vortexed every 2 to 3 minutes to assist dissolving. |
| | Isopropanol was not added prior to binding | Ensure that 1 gel volume of Isopropanol is added to the melted gel slice prior to binding to the column. |
| | The appropriate amount of ethanol was not added to the **Wash Solution A** | The **Wash Solution A** has been specifically designed to contain the appropriate amount of components. Ensure that the **Wash Solution A** was prepared with the correct amount of 96-100% ethanol. |
| | Binding of DNA to the column was inefficient | Binding of the DNA is dependent on both pH and salt concentration. Ensure that an appropriate amount of **Binding Buffer G** was used for the weight of the gel slice. |
| | **Binding Buffer G** was not completely removed in the wash step | Traces of salt left on the column from the binding step may interfere with the elution of the DNA. Ensure that the column is washed with the **Wash Solution A**. |
| | Proper **Elution Buffer B** was not used | The provided **Elution Buffer B** has been optimized for high elution recoveries. If water or TE buffer is used instead, ensure the pH is around 8. |
| | **Elution Buffer B** was not placed directly onto the column bed | It is important that the **Elution Buffer B** be placed directly onto the column bed, as this helps to increase recovery by ensuring an even passing of the buffer through the column. Do not pipette the **Elution Buffer B** onto the side of the column. |
| DNA does not perform well in downstream applications | Incomplete removal of **Wash Solution A** | Ensure that the column is spun for 2 minutes during the wash step, in order to completely dry the column. Traces of **Wash Solution A** may remain in the eluted sample otherwise, and interfere with subsequent enzymatic reactions. |
| | DNA was not washed with the provided **Wash Solution A** | Traces of salt from the binding step may remain in the sample if the column is not washed with **Wash Solution A**. Salt may interfere with downstream applications, and thus must be washed from the column. |

## Appendix 1

**Separation of DNA in Gels Containing Different Concentrations of Agarose**

| Amount of Agarose (Percentage) | Efficient Range of Separation of Linear DNA Molecules (kbp) |
|---|---|
| 0.3 | 5 - 60 |
| 0.6 | 1 - 20 |
| 0.7 | 0.8 - 10 |
| 0.9 | 0.5 - 7 |
| 1.2 | 0.4 - 6 |
| 1.5 | 0.2 - 3 |
| 2.0 | 0.1 - 2 |

## Appendix 2

**Time Required to Melt 100 mg of Gel Slices of Varying Agarose Concentrations at 55°C**

| Percent Agarose | Three (3) Volumes Binding Buffer G | Six (6) Volumes Binding Buffer G |
|---|---|---|
| 1 % | 4 minutes | 4 minutes |
| 2% | 4 minutes | 4 minutes |
| 3 % | NR | 8 minutes |
| 4 % | NR | 10 minutes |

NR = Not Recommended. Norgen does not recommend the use of 3 volumes of Binding Buffer G for greater than 2% gels since resulting melted gel slice is viscous and will hinder the flow of solutions through spin columns.

| Related Products | Product # |
|---|---|
| Norgen PCR Purification Kit | 14400 |
| Norgen Enzymatic Reaction Clean-Up Kit | 19900 |

**Technical Support**
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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