

# **Gel Extraction & PCR Cleanup System**

**Catalog Number 500-100**

***Ana-Gen Technologies, Inc.***

**Phone: (404)223-5090 • Fax: (404)601-0747 • <http://www.ana-gen.com>**

---

**Table of Contents:**

<b>I. Kit Components</b> .....	<b>1</b>
<b>II. Introduction</b> .....	<b>2</b>
<b>III. Purification of DNA Fragments from Agarose Gels</b> .....	<b>2</b>
<b>IV. Purification of DNA Fragments from Acrylamide Gels</b> .....	<b>4</b>
<b>V. Removal of Unincorporated PCR primers</b> .....	<b>5</b>
<b>VI. Purification of DNA fragments from enzymatic reactions</b> .....	<b>6</b>
<b>VII. Troubleshooting</b> .....	<b>7</b>
<b>VIII. Product Warranty</b> .....	<b>9</b>

---

100 purifications (Cat. no. 500-100)

### **I. Kit Components**

All Buffers and kit components should be stored at room temperature. When stored under ambient conditions, all kit components will retain full activity for a period of at least 9 months.

- **100 Scorpion spin columns for gel extraction (orange)**

The binding capacity of each column is approximately 10 µg.

**Note:** The orange color designates this as a gel extraction spin column.

- **100 x 1.5 ml graduated, capped microcentrifuge tubes** for estimating agarose volumes
- **100 x 2 ml collection tubes**
- **Instructions**
- **Gel Extraction Buffers 1-3**

#### **Gel Extraction Buffer 1 (100 ml)**

- Proprietary formulation.
- Potential acidic irritant (pH 4.5): contains a mix of salts and nontoxic additives
- Always wear eye protection and gloves when handling.

#### **Gel Extraction Buffer 2 (20 ml 5x concentrate)**

- 10 mM Tris-Cl, pH 7.5 (final)
- 80% ethanol (final)
- Nontoxic and noncaustic

**IMPORTANT:** The customer is required to add 80 ml of 95-100% ethanol to the Buffer 2 concentrate to generate the working wash buffer. Either denatured or nondenatured ethanol may be used.

#### **Gel Extraction Buffer 3 (20 ml)**

- 12 mM Tris-Cl, pH 8.5
- Nontoxic and noncaustic

## II. Introduction

The Scorpion Gel Extraction & PCR Cleanup System is a versatile kit which can be used to purify DNA fragments under a wide variety of conditions for downstream applications such as ligations, sequencing, restrictions, enzymatic modification, etc. The protocols contained in this manual describe the purification of DNA fragments from agarose and acrylamide gels, PCR and other enzymatic reactions.

The Scorpion spin columns are compatible with any vacuum manifold which has standard luer fittings. These columns are also fully adaptable to other manufacturers' protocols, provided that Ana-Gen reagents are used. When using a vacuum manifold, simply substitute the Scorpion spin columns and buffers.

## III. Agarose Gel Extraction Protocol

***Please read all steps carefully before proceeding.***

This protocol is designed to purify DNA fragments from agarose gels comprised of up to 2% agarose. This protocol will work well with either standard or low melting temperature agaroses, using either TAE or TBE as electrolytes. This procedure will routinely produce yields of  $\geq 75\%$  when isolating DNA fragments over a size range of approximately 100 bp up to 10 kb. Special additives provide greater uniformity of yield when purifying fragments of different lengths and minimize the carryover of residual agarose. This kit contains sufficient reagents to process 100, 2% agarose gel slices of up to 250  $\mu\text{l}$  in volume, each.

- 1. Excise the DNA fragment band from the gel (up to 2% agarose), minimizing the amount of agarose.**
- 2. Place the gel slice into one of the *capped* graduated 1.5 ml microcentrifuge tubes (provided) and centrifuge for 30 seconds at top speed ( $\geq 12,000 \times g$ ).** This will compress the gel in the bottom of the tube.
- 3. From the graduations on the side of the tube, estimate the approximate volume of the gel.**  
**Note:** It is not necessary to *precisely* determine the gel volume. An estimation will suffice.
- 4. Add three gel volumes of Gel Extraction Buffer 1.**  
For example, if the approximate gel volume was 200  $\mu\text{l}$ , add 600  $\mu\text{l}$  of Buffer 1.
- 5. Heat the solution for 10 minutes at 60°C.**  
Inverting the tube or vortexing a few times during this period will accelerate solubilization of the gel.
- 6. Pipet or pour the liquified gel solution into the Scorpion spin column.** The spin column should be seated in a 2 ml collection tube.
- 7. Bind the DNA fragment to the spin column membrane with a low speed spin.**  
Centrifuge the spin column for **1 minute at 3,000-4,000 rpm (approx. 1,000  $\times g$ )**. The binding of the DNA fragment to the spin column membrane is dependent upon a diffusive interaction between the fragment and the surface of the fibers comprising the membrane. This interaction is facilitated by moving the solution containing the fragment through the membrane at a modest speed.

At the end of the spin, check to ensure that no solution remains in the spin column. Discard the solution in the collection tube but save the tube for reuse in the next step.

**Note:** Depending upon a variety of factors, such as the size of the gel slice, the percent agarose, etc., there may occasionally be traces of agarose which are retained by the spin column and later copurify with the DNA fragment. This small amount of agarose is generally innocuous in most downstream reactions and applications. However, there are some applications, such as microinjection where traces of agarose may have an adverse effect. In those cases, we recommend performing an additional “**warm wash**” with Buffer 1 by loading **400 µl of prewarmed (45 °C) Gel Extraction Buffer 1** and spinning for **1 minute at top speed ( $\geq 12,000 \times g$ )**. This should eliminate all traces of agarose. Extra Gel Buffer 1 is included to accommodate the occasional addition of this step. If this optional wash step is performed *routinely*, additional Buffer 1 can be purchased from Ana-Gen (*please inquire*).

**8. Wash the spin column with 750 µl of Gel Extraction Buffer 2.**

Add 750 µl of Buffer 2 to the spin column and centrifuge for **1 minute at 3,000-4,000 rpm**.

**9. Empty the wash buffer from the collection tube and centrifuge the spin column for 1 minute at top speed ( $\geq 12,000 \times g$ ).**

This high-speed spin is used to completely expel any remaining wash buffer which may be residing within the spin column.

**10. Transfer the spin column to a clean 1.5 ml microcentrifuge tube (supplied by customer).**

Check to ensure that no solution remains within the spin column. Remove the spin column from the collection tube and, if necessary, blot the luer end with a clean absorbent tissue, such as a kimwipe to remove any traces of wash buffer. Place the spin column into a new microcentrifuge tube.

**Note:** Do not use one of the 2 ml kit collection tubes for this.

**11. Elute the bound DNA fragment with 60 µl of Gel Extraction Buffer 3.**

Pipet 60 µl (standard eluent volume) onto the membrane at the base of the spin column. Be careful not to puncture the membrane with the pipet tip. **Allow 1 minute for detachment of the DNA fragment from the chromatographic membrane before centrifugation. Spin for 1 minute at top speed ( $\geq 12,000 \times g$ )** to completely expel all of the material.

**Note:** An approach which can be used to maximize DNA fragment recovery is the use of a relatively large volume of eluent (e.g., 100 µl) followed by sample concentration (if necessary) with a speed-vac. Extra Buffer 3 is supplied to accommodate this. This approach may increase the yield by 10-20% with some fragments.

**Note:** TE or deionized water can also be used as eluents, provided that the pH is 8.0-8.5. However, the chemical stability of DNA is diminished when stored in pure (nonbuffered) water for extended periods. Some investigators have reported the inhibition of enzymatic reactions, such as PCR, when even small amounts (0.5-1 mM) of EDTA are present. Speed-vac concentration of plasmids eluted in TE may concentrate the EDTA to a level which may interfere with subsequent enzymatic reactions.

## IV. Purification of DNA Fragments from Acrylamide Gels

### ***Please read all steps carefully before proceeding***

This procedure is designed to rapidly recover DNA fragments from acrylamide gels with a significant reduction in loss compared to other methods. The percent yield is generally lower from acrylamide gels than from agarose gels.

- 1. After excising the acrylamide gel slice containing the DNA fragment of interest, blot it gently with an absorbent tissue (e.g., kimwipe) until it become slightly tacky.**  
Transfer it to one of the capped, graduated microcentrifuge tubes provided in the kit.
- 2. Using a small spatula, crush the acrylamide slice by repeatedly pressing it against the wall of the tube.** Continue to crush until the acrylamide forms a spongy ball.
- 3. Centrifuge for 30 seconds at top speed ( $\geq 12,000 \times g$ ) to compress the crushed acrylamide in the bottom of the tube.**
- 4. From the graduations on the side of the tube, estimate the approximate volume of the acrylamide.**  
**Note:** It is not necessary to *precisely* determine the exact volume. An estimation will suffice.
- 5. Add two gel volumes of deionized water and vortex vigorously for 30 seconds.**  
**Note:** Sometimes yield can be improved somewhat by allowing the hydrated acrylamide to sit overnight at 4°C.
- 6. Add two gel volumes of Gel Extraction Buffer 1 and vortex vigorously for 30 seconds.**
- 7. Centrifuge for 1 minute at top speed ( $\geq 12,000 \times g$ ).**
- 8. Pipet off as much solution as possible.**  
The pipette tip can be inserted down through the crushed acrylamide pellet to the bottom of the tube to maximize recovery.
- 9. Load the solution into a Scorpion spin column.** The spin column should be seated in one of the 2 ml collection tubes provided in the kit.  
**Note:** Transfer of acrylamide to the spin column is irrelevant.
- 10. Bind the DNA fragment to the spin column membrane with a low speed spin.**  
Centrifuge the spin column for **1 minute at 3,000-4,000 rpm (approx. 1,000 x g)**.
- 11. Wash the spin column with 750  $\mu$ l of Gel Extraction Buffer 2.**  
Add 750  $\mu$ l of Buffer B to the spin column and centrifuge for **1 minute at 3,000-4,000 rpm**.
- 12. Empty the wash buffer from the collection tube and centrifuge the spin column for 1 minute at top speed ( $\geq 12,000 \times g$ ).**  
This high-speed spin is used to completely expel any remaining wash buffer which may be residing within the spin column.
- 13. Transfer the spin column to a clean 1.5 ml microcentrifuge tube (supplied by customer).**  
Check to ensure that no solution remains within the spin column. Remove the spin column from the collection tube and, if necessary, blot the luer end with a clean absorbent tissue,

such as a kimwipe to remove any traces of wash buffer. Place the spin column into a new microcentrifuge tube. **Note:** Do not use one of the 2 ml kit collection tubes for this

**14. Elute the bound DNA fragment with 60  $\mu$ l of Gel Extraction Buffer 3.**

Pipet 60  $\mu$ l (standard eluent volume) onto the membrane at the base of the spin column. Be careful not to puncture the membrane with the pipet tip. **Allow 1 minute for detachment of the DNA fragment from the chromatographic membrane before centrifugation. Spin for 1 minute at top speed ( $\geq 12,000 \times g$ )** to completely expel all of the material.

## **V. Removal of Unincorporated PCR primers (PCR Reaction Cleanup)**

### ***Please read all steps carefully before proceeding***

Although this kit is configured primarily to purify DNA fragments from agarose gels, it can also be used to remove unincorporated primers from PCR\* products. The following procedure is designed to remove residual PCR primers of up to 30 nt in length, DNA polymerase and salts following PCR amplification. It should provide routine yields of approximately  $\geq 75\%$  when purifying DNA fragments of  $\geq 100$  bp in length. This procedure is not recommended for primers longer than 30 nt. When primers  $>30$ nt must be removed, we recommend the Gel Extraction Procedure (pg. 2)

**1. Increase the volume of the PCR to 100  $\mu$ l using deionized water.**

**For example:** If the volume of the PCR is 50  $\mu$ l, add 50  $\mu$ l of deionized water to generate a diluted PCR with a final volume of 100  $\mu$ l.

**Note:** If an oil overlay has been used, pipet the sample out from underneath the overlay and transfer into a clean microcentrifuge tube. Significant oil contamination may alter the uniform flow of the solutions through the spin column membrane, resulting in suboptimal binding and elution of the DNA fragments.

**2. Add 100  $\mu$ l of Gel Extraction Buffer 1 to the diluted reaction.**

This is intended to establish a salt environment in which PCR fragments  $\geq 100$  bp will bind to the Scorpion spin column but primers of  $\leq 30$  nt will not bind.

**Note:** Please be aware that there are no *absolute* size cutoffs established in this procedure. PCR fragments somewhat shorter than 100 bp may bind with adequate affinity to be retained and primers somewhat longer than 30 nt will also be quantitatively removed. The figures above are provided simply because they establish a set of parameters in which reliable performance can be expected.

**3. Load the solution into a Scorpion spin column,** seated in one of the 2 ml collection tubes provided in the kit.

**4. Bind the DNA fragment to the spin column membrane with a low speed spin.**

Centrifuge the spin column for **1 minute at 3,000-4,000 rpm (approx. 1,000  $\times g$ )**.

**5. Wash the spin column with 750  $\mu$ l of Gel Extraction Buffer 2.**

Add 750  $\mu$ l of Buffer 2 to the spin column and centrifuge for **1 minute at 3,000-4,000 rpm**.

**6. Empty the wash buffer from the collection tube and centrifuge the spin column for 1 minute at top speed ( $\geq 12,000 \times g$ ).**

This high-speed spin is used to completely expel any remaining wash buffer which may be residing within the spin column.

**7. Transfer the spin column to a clean 1.5 ml microcentrifuge tube (supplied by customer).**

Check to ensure that no solution remains within the spin column. Remove the spin column from the collection tube and, if necessary, blot the luer end with a clean absorbent tissue,

such as a kimwipe to remove any traces of wash buffer. Place the spin column into a new microcentrifuge tube.

**Note:** Do not use one of the 2 ml kit collection tubes for this.

**8. Elute the bound DNA fragment with 60  $\mu$ l of Gel Extraction Buffer 3.**

Pipet 60  $\mu$ l (standard eluent volume) onto the membrane at the base of the spin column. Be careful not to puncture the membrane with the pipet tip. **Allow 1 minute for detachment of the DNA fragment from the chromatographic membrane before centrifugation. Spin for 1 minute at top speed ( $\geq 12,000 \times g$ )** to completely expel all of the material.

**VI. Purification of DNA fragments from enzymatic reactions.**

This protocol will allow the purification of double-stranded DNA fragments (and plasmids) of approximately 100 bp-10kbp from various enzymatic reactions, such as restriction digests.

**1. Increase the volume of the enzymatic reaction to 100  $\mu$ l using deionized water.**

**For example:** If the reaction volume is 50  $\mu$ l, add 50  $\mu$ l of deionized water to generate a final volume of 100  $\mu$ l.

**2. Add 100  $\mu$ l of Gel Extraction Buffer 1 to the diluted reaction.**

**Note:** For DNA fragments <100 bp in length, recovery can be improved by increasing the amount of Buffer 1. Increasing the volume to 200  $\mu$ l may allow the recovery of a sufficient amount of material for further work.

**3. Load the solution into a Scorpion spin column.**

**4. Bind the DNA fragment(s) to the spin column membrane with a low speed spin.**

Centrifuge the spin column for **1 minute at 3,000-4,000 rpm (approx. 1,000  $\times g$ )**.

**5. Wash the spin column with 750  $\mu$ l of Gel Extraction Buffer 2.**

Add 750  $\mu$ l of Buffer 2 to the spin column and centrifuge for **1 minute at 3,000-4,000 rpm**.

**6. Empty the wash buffer from the collection tube and centrifuge the spin column for 1 minute at top speed ( $\geq 12,000 \times g$ ).**

This high-speed spin is used to completely expel any remaining wash buffer which may be residing within the spin column.

**7. Transfer the spin column to a clean 1.5 ml microcentrifuge tube (supplied by customer).**

Check to ensure that no solution remains within the spin column. Remove the spin column from the collection tube and, if necessary, blot the luer end with a clean absorbent tissue, such as a kimwipe to remove any traces of wash buffer. Place the spin column into a new microcentrifuge tube.

**Note:** Do not use one of the 2 ml kit collection tubes for this.

**8. Elute the bound DNA fragment with 60  $\mu$ l of Gel Extraction Buffer 3.**

Pipet 60  $\mu$ l (standard eluent volume) onto the membrane at the base of the spin column. Be careful not to puncture the membrane with the pipet tip. **Allow 1 minute for detachment of the DNA fragment from the chromatographic membrane before centrifugation. Spin for 1 minute at top speed ( $\geq 12,000 \times g$ )** to completely expel all of the material.

## VIII. Troubleshooting

### 1. Low/no apparent yield

Fragments isolated from gels may be present in only limited mass amount (<100 ng) making quantitation difficult without sacrificing most of the purified sample. In those cases where there is a significant loss of material, the following measure can be quite useful for routinely increasing the yield.

- a. Use Buffer 3 warmed to 40-45 °C for elution.
- b. Increase the volume of eluent to 100-200 µl and precipitate fragment with a glycogen carrier to concentrate.

### 2. DNA fragment fails to perform well in enzymatic reactions.

When reactions with experimentally-derived DNA fragments fail to perform as anticipated, an accompanying control reaction will immediately allow one to determine if the problem lies with the fragment or with some other constituent of the reaction, such as the enzyme or reaction buffer.

When the appropriate control reaction verifies a problematic fragment, the problem will almost always be attributable to one of the following:

- insufficient quantity of fragment
  - contamination of fragment with residual chaotrope, ethanol or excessive agarose
- To eliminate residual chaotrope or ethanol, carry out an ethanol precipitation as follows:

- a. Add 1 µg of glycogen carrier.
- b. Add 1/3 volume 7.5 M ammonium acetate.
- c. Add two volumes 100% ethanol.

Chill on dry ice or at -80 °C for 30 minutes. Centrifuge for 20 minutes at  $\geq 12,000 \times g$ . Wash 2x with ice-cold 70% ethanol, air-dry and resuspend in deionized water.

To eliminate residual agarose, always carry out the chaotrope wash described in Step 7 (pg. 2).

To avoid these problems in the future, introduce the following modifications to the basic protocol:

- Increase the amount of Buffer 1 from 3 to 4 volumes with gels of up to 2%.
- Always perform the chaotrope wash with Buffer 1, described in Step 7., pg. 2.
- Spin for 2 minutes at top speed ( $> 12,000 \times g$ ) to remove all traces of Buffer 1 and residual agarose.
- Spin for 2 minutes at top speed ( $\geq 12,000 \times g$ ) to remove all traces of Buffer 2 prior to elution.

### 3. Spin column clogs

This is a clear indication that the agarose was incompletely solubilized before loading into the spin column. This is likely to occur if the volume of agarose is grossly underestimated or if the gel slice and Buffer 1 solution was not incubated at a sufficiently high temperature. When pipetting the solubilized agarose solution, always watch the flow of the solution into the pipette tip. The solution should be nonviscous and flow smoothly through the pipette tip orifice. Clogging of the pipette tip is a clear indication that the gel is not fully solubilized. Additional measures which can be taken:

- Increase the gel solubilization temperature to 70 °C.
- Increase the amount of Buffer 1 from 3 to 4 volumes with gels of up to 2%.
- Use frequent vortexing or inversion during period of heating.

### 4. Fragment floats out of well when loaded on an agarose gel.

This is due to residual ethanol from Buffer 2. If the fragment must be evaluated on a gel, load the sample into the well without a buffer overlayer, but be sure the buffer on each end is even with the top of the gel. Run the sample into the gel for 10 minutes and then cover with buffer as usual.

### **VIII. Product Warranty**

Ana-Gen Technologies (Ana-Gen) products are sold for research purposes only. Each production lot is subjected to appropriate quality assurance procedures and is warranted to perform as indicated when handled, stored and used according to the manufacturer's instructions. Please report any problems to Ana-Gen immediately by calling (404)223-5090. By using this product, the customer acknowledges and agrees that any liability which Ana-Gen incurs to the customer is at the discretion of Ana-Gen and is limited to either the replacement of the product or in certain cases, to a credit for the price paid to Ana-Gen for the product, including applicable shipping and handling costs. The customer further agrees that any liability assumed by, or any remedial action taken by Ana-Gen is contingent upon the customer providing full details of the nature of the problem and the circumstances of use to Ana-Gen, including return of the product to Ana-Gen for testing. Any remedial measures taken by Ana-Gen will occur only AFTER testing and verification of malperformance by Ana-Gen of the product in question. On occasion, Ana-Gen will, at the customer's request, provide "custom" protocols which are intended to allow a kit or product to be employed in a particular set of unconventional circumstances, as determined by the customer. Ana-Gen makes no guarantee concerning the performance of its kits or products when used in custom protocols or any protocols which depart from the specific instructions provided in a kit. ***Ana-Gen will not, under any circumstances provide the specific composition of solutions, reagents or any kit component which it regards and/or has designated as "proprietary."***

\*The Polymerase Chain Reaction (PCR) is a process covered by patents owned by Hoffmann-LaRoche

---

**Ana-Gen Technologies, Inc.**  
2880 N. Berkeley Lake Road  
Suite 3  
Duluth, GA 30022  
Phone: (404)223-5090 • Fax: (404)601-0747 • <http://www.ana-gen.com>