Product Manual

PCR & Gel Clean-up Kit

Catalog #: ENZ-GEN100

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Product Manual

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Please read entire booklet before proceeding with the assay.

Please contact Enzo Life Sciences Technical Support if necessary.

SAFETY WARNINGS & PRECAUTIONS

Wear gloves and goggles and follow the safety instructions.

MATERIALS SUPPLIED

	20 preps	50 preps	200 preps
Component	**ENZ-GEN100-0020**	**ENZ-GEN100-0050**	**ENZ-GEN100-0200**
Binding Buffer (w/ pH indicator)	20 mL	50 mL	4 x 50 mL
5X Wash Buffer	8 mL	20 mL	4 x 20 mL
Elution Buffer	4 mL	10 mL	4 x 10 mL
PCR & Gel Clean-up Columns (yellow ring)	20	50	200
Collection Tubes (2 mL)	20	50	200

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

Consumables

- 96-100% ethanol
- 1.5 mL microcentrifuge tubes
- Disposable pipette tips
- Optional: TE buffer (10 mM Tris, 1mM EDTA, pH 8.0)
- Optional: PCR grade water

Equipment

- Manual pipettes
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)
- Optional: Heating block

PRINCIPLE

The PCR & Gel Clean-up Kit is designed to purify DNA fragments from enzymatic reactions, such as PCR, and from agarose gels.

Binding Buffer is added to a PCR reaction or agarose gel slice and the mixture is subsequently loaded directly onto PCR & Gel Clean-up Columns. The buffer contains chaotropic salts that allow DNA to bind to the silica membrane in the column. Salts, enzymes, and other soluble components are washed away with an ethanol based Wash Buffer. Purified DNA is eluted under low salt conditions using a slightly alkaline Elution Buffer (5 mM Tris-HCl, pH 8.5).

The PCR & Gel Clean-up Kits can be used downstream of DNA labeling with Enzo's CYTAG® CGH labeling Kit (Prod. No. ENZ-42671) and are included with the CYTAG® TotalCGH labeling kit (Prod. No. ENZ-42674) to clean up excess nucleotides and enzymes. The "DNA Purification After CGH Labeling Reactions" protocol should be used for this application (see page 9).

PCR & Gel Clean-up Kit Specifications

- PCR & Gel Clean-up Kits are designed for DNA purification from TAE/TBE agarose gels and for the direct purification of PCR products.
- PCR & Gel Clean-up buffers are formulated to completely remove primers from PCR reactions. DNA fragments greater than 50 bp still remain bound and are purified with high efficiency.
- PCR & Gel Clean-up Kits will effectively purify DNA fragments from detergent-rich PCR reaction buffers.
- DNA absorption to the membrane is pH-dependent. TAE standard gels or reaction mixtures with pH 6-8 should be used for best results.
- Both standard and low melting agarose gels can be used.
- PCR and Gel purified DNA fragments are ready to use in downstream applications like automated fluorescent DNA sequencing, PCR, ligation reactions, genomic hybridizations, or other types of enzymatic manipulation.

PCR & Gel Clean-up Kits have been validated for use with Enzo's CYTAG® CGH labeling kit (Prod. No. ENZ-42671) and are included with the CYTAG® TotalCGH labeling kit (Prod. No. ENZ-42674).

PCR & Gel Clean-Up Kit Specifications	
DNA fragments from agarose gels	50 bp–20 kbp
Elution volume	15-30 µL
Binding capacity	25 µg
Time/prep	10 minutes for 6 preparations

Removal of small DNA fragments and primer-dimers

The PCR & Gel Clean-up Kit is specially formulated to remove unused, single stranded primers while effectively purifying PCR products down to 50 bp. In some cases, a PCR reaction may yield unwanted small double stranded fragments, such as primer-dimers or small PCR products resulting from unspecific annealing. The PCR & Gel Clean-up Kit offers a simple method to remove these products that can interfere with downstream sequencing or cloning applications.

By simply diluting Binding Buffer with sterile water, you can decrease the ability of small DNA fragments to bind to the membrane without compromising larger fragment recovery. A simple dilution series should be tested, ranging from 1:1-1:9 (Binding Buffer 1:H_2O) in order to determine the appropriate cut off range for your reaction. As you approach the 1:9 dilution, the larger fragment recovery will sequentially decrease as well.

Rule of Thumb: The smaller the fragment you wish to exclude, the less you will need to dilute the Binding Buffer.

Elution procedural tips

DNA should be eluted using Elution Buffer. If necessary, sterile water or other low salt elution buffers may be used, however the pH must be in the range of 7.0-8.5 for optimal recovery.

Typical recovery of 70-95% can be obtained with DNA fragments between 50 bp -10 kbp with an elution volume of 15 µL. For larger amounts of DNA (5-15 µg of DNA; from PCR reactions > 100 µL or gel slices > 200 mg), two elutions with 30 µL of PCR Elution Buffer is recommended.

Pre-warmed Elution Buffer can improve the yields of larger fragments (> 5-10 kbp). Add pre-warmed Elution Buffer (70°C) to the membrane, and incubate for 1-2 minutes, then centrifuge as directed in the standard protocol.

DNA Recovery with PCR & Gel Clean-up Kit		
Fragment length	**Elution volume**	**Recovery**
65 bp	15 µL 25 µL 50 µL 100 µL	85% 90% 95% 95%
400 bp	15 µL 25 µL 50 µL 100 µL	85% 95% 100% 100%
500 bp	15 µL 25 µL 50 µL 100 µl	85% 90% 95% 95%
700 bp	15 µL 25 µL 50 µL 100 µL	85% 85% 90% 95%

BINDING BUFFER WITH PH INDICATOR

The optimal pH to bind small DNA fragments to the silica membrane is ~5-6. The Binding Buffer is buffered to maintain this pH but to ensure that the pH is correct, a pH indicator has been added. The pH indicator does not interfere with DNA binding and is completely removed during the purification. The yellow color is also beneficial for gel extractions, for easy identification of undissolved agarose pieces. For restoring the correct pH conditions, add more Binding Buffer or 4M sodium acetate, pH 5.0, or small amounts of HCl until the color switches back to yellow.

Yellow – correct conditions
Green – pH slightly too high, adjustment recommended
Blue – pH too high, adjustment necessary

STORAGE AND PREPARATION OF SOLUTIONS

Note: Binding Buffer contains chaotropic salt. Wear gloves and goggles!

PCR & Gel Clean-up Kit components should be stored at room temperature and are stable for up to one year.

The following should be prepared before starting any Enzo PCR purification or gel extraction protocols:

1x Wash Buffer - Add 96-100% ethanol to 5X Wash Buffer Concentrate. For example, for ENZ-GEN100-0020 (20 preparations), add 32 mL of 96-100% ethanol to the 8 mL 5x Wash Buffer to make a 40 mL 1X Wash Buffer.

Follow the guideline below to prepare a 1X Wash Buffer:

	20 preps	**50 preps**	**200 preps**
Component	**ENZ-GEN100-0020**	**ENZ-GEN100-0050**	**ENZ-GEN100-0200**
Wash Buffer 5X	8 mL	20 mL	4 x 20 mL
Amount of 96-100% Ethanol to add	32 mL	80 mL	4 x 80 mL

PCR CLEAN-UP

1. **Adjust DNA binding conditions**
   - Mix 1 volume of sample with 2 volumes of Binding Buffer (e.g. mix 100 µL PCR reaction and 200 µL Binding Buffer).
   - For sample volumes < 50 µL, adjust the volume of the reaction mix to 50-100 µL using TE buffer or water.
   - Dilutions of Binding Buffer may be used for removal of primers or nonspecific products, see page 4.
2. **Bind DNA**
   - Place a PCR & Gel Clean-up Column into a 2 mL Collection Tube and load the sample.
   - Centrifuge for 60 seconds at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.
3. **Wash silica membrane**
   - Add 650 µL 1X Wash Buffer. Centrifuge for 60 seconds at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.
   - Optional: To prevent salt carryover for sensitive procedures, add an additional 300 µL 1X Wash Buffer and repeat wash step 3. In addition, incubation with the 1X Wash Buffer at room temperature for 1 minute before centrifugation will remove additional salt.
4. **Dry silica membrane**
   - Centrifuge for 2 minutes at 11,000 x g to remove Wash Buffer. The tip of the spin column should not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.
5. **Elute DNA**
   - Place the PCR & Gel Clean-up Column into a clean 1.5 mL microcentrifuge tube (not provided). Add 15-30 µL Elution Buffer and incubate at room temperature for 1 minute to increase the yield of eluted DNA.
   - Centrifuge for 1 minute at 11,000 x g.
   - Optional: Multiple elution steps and the use of pre-warmed Elution Buffer (70°C) can be used to increase the yield of larger fragments (> 1000 bp).

DNA EXTRACTION FROM AGAROSE GELS

1. **Excise DNA fragment / Solubilize gel slice**
    - Excise the DNA fragment from an agarose gel using a clean scalpel. Excise the gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer to a clean tube.
    - For each 100 mg of agarose gel, add 200 µL Binding Buffer.
    - For gels containing > 2% agarose, double the volume of Binding Buffer. Max gel slice size is 400 mg for normal gel (<2%) or 200 mg for high percentage gel (> 2%).
    - Incubate sample at 50°C until gel slice is fully dissolved (5-10 minutes). Vortex the sample briefly every 2-3 minutes during incubation.
2. **Bind DNA**
    - Place a PCR & Gel Clean-up Column (yellow ring) into a 2 mL Collection Tube and load the sample.
    - Centrifuge for 60 seconds at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.
3. **Wash silica membrane**
    - Add 650 µL 1X Wash Buffer. Centrifuge for 60 seconds at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.
    - Optional: To prevent salt carryover for sensitive procedures, add an additional 300 µL 1X Wash Buffer and repeat wash step 3. In addition, incubation with the 1X Wash Buffer at room temperature for 1 minute before centrifugation will remove additional salt.
4. **Dry silica membrane**
    - Centrifuge for 2 minutes at 11,000 x g with the lid open to remove the Wash Buffer. The tip of the spin column should not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.
5. **DNA Elution**
    - Place the PCR & Gel Clean-up Column into a clean 1.5 mL microcentrifuge tube (not provided). Add 15-30 µL Elution Buffer and incubate at room temperature for 1 minute to increase the yield of eluted DNA.
    - Centrifuge for 1 minute at 11,000 x g.
    - Multiple elution steps and the use of pre-warmed Elution Buffer (70°C) can be used to increase the yield of larger fragments (> 1000 bp).

DNA PURIFICATION AFTER CGH LABELING REACTIONS

This protocol is to be followed after labeling of your DNA sample with Enzo's CYTAG® CGH labeling kit (Prod. No. ENZ-42671), or CYTAG® TotalCGH labeling kit (Prod. No. ENZ-42674). Purify each labeling reaction in separate columns (combining the labeled sample and labeled reference DNA is not recommended prior to purification). For reference, the binding capacity of the columns for *labeled* DNA is approximately 10 µg.

1. Bind DNA to the column

- For each sample, place a PCR & Gel Clean-up Column into a 2 mL Collection Tube
- Mix labeled sample with 2 volumes (100 µL or 110 µL, if Stop Buffer was used) of Binding Buffer by pipetting up and down a few times, one sample per tube at a time.
- Immediately add to a PCR & Gel Clean-up Column.
- Follow same steps for the remainder of samples.
- Centrifuge for 30 sec at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.

2. Wash silica membrane

- Add 650 µL 1X Wash Buffer.
- Centrifuge for 30 seconds at 11,000 x g. Discard flow-through and place the PCR & Gel Clean-up Column back into the Collection Tube.
- Add an additional 650 µL Wash Buffer and repeat previous step.

3. Dry silica membrane

- Centrifuge for 2 minutes at 11,000 x g with the lid open to remove Wash Buffer. The tip of the spin column should not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.

4. Elute DNA

- Place the PCR & Gel Clean-up Column into a clean 1.5 mL microcentrifuge tube (not provided). Add 25 µL Elution Buffer and incubate at room temperature for 1 minute to increase the yield.
- Centrifuge for 1 minute at 11,000 x g.
- Add another 25 µL Elution Buffer and repeat previous step to obtain a total of volume 50 µL.

NOTE: Based upon the requirements of your hybridization platform, volume reduction may be required.

TROUBLESHOOTING

Problem	Suggestion
Incomplete dissolving of agarose slices	*High amount of agarose* • Use doubled volumes of Binding Buffer for highly concentrated and/or low melting point agarose gels. *Time and temperature* • Check incubation temperature. Depending on the weight of the gel slice, incubation in Binding Buffer can continue up to 20 min. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be crushed before adding the Binding Buffer.
Low DNA yield	*Reagents not applied properly* • Add indicated volume of 96-100% ethanol to Wash Buffer Concentrate and mix well before use. *Incompletely dissolved gel slice* • Increase time or add another two volumes of Wash Buffer and vortex the tube every 2 minutes during incubation at 50°C. Small pieces of gel may be difficult to see and contain DNA that will be lost for purification. *Not enough elution buffer* • For larger amounts of DNA (> 5 µg), elute twice with 30-50 µL elution buffer each time. *Isolation of large DNA fragments* • Preheat Elution Buffer to 70°C, and incubate on the silica membrane at room temperature for 2 min before centrifugation.
Appearance of additional bands on agarose gel	*Appearance of additional bands on agarose gel* • When eluting with water or using agarose with low ion content, denatured (single-stranded) DNA can be present. To re-anneal the DNA, add all components of the subsequent enzymatic reaction omitting the enzyme. Incubate at 95°C for 2 min and let the mixture cool slowly to room temperature (at this step the DNA re-anneals). Add the enzyme and continue with your downstream application.

Poor performance in sequencing, restriction or ligation reactions	*Carry-over of chaotropic salts* • Perform a second washing step with Wash Buffer and incubate at room temperature for 1 minute prior to centrifugation. *Elution of DNA with outside buffers, e.g. TE buffer (Tris/EDTA)* • EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Elution Buffer or water, pH 7.0–8.5. *Not enough DNA used for sequencing reaction* • Quantify DNA before setting up sequencing reactions.
Poor performance in NanoDrop® Spectrophotometer Analysis or Agilent's Bioanalyzer	*Carry-over of trace amounts of silica particles* • NanoDrop® Spectrophotometer technology is very sensitive to any particles included in the sample material. To pellet the silica particles centrifuge > 2 min at 11,000 x *g* and take the supernatant for further use.
Strong absorbance at 230 nm	*Carry-over of chaotropic salts* • Perform a second washing step with Wash Buffer and incubate at room temperature for 1 minute prior to centrifugation.

REFERENCES

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

NOTES

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