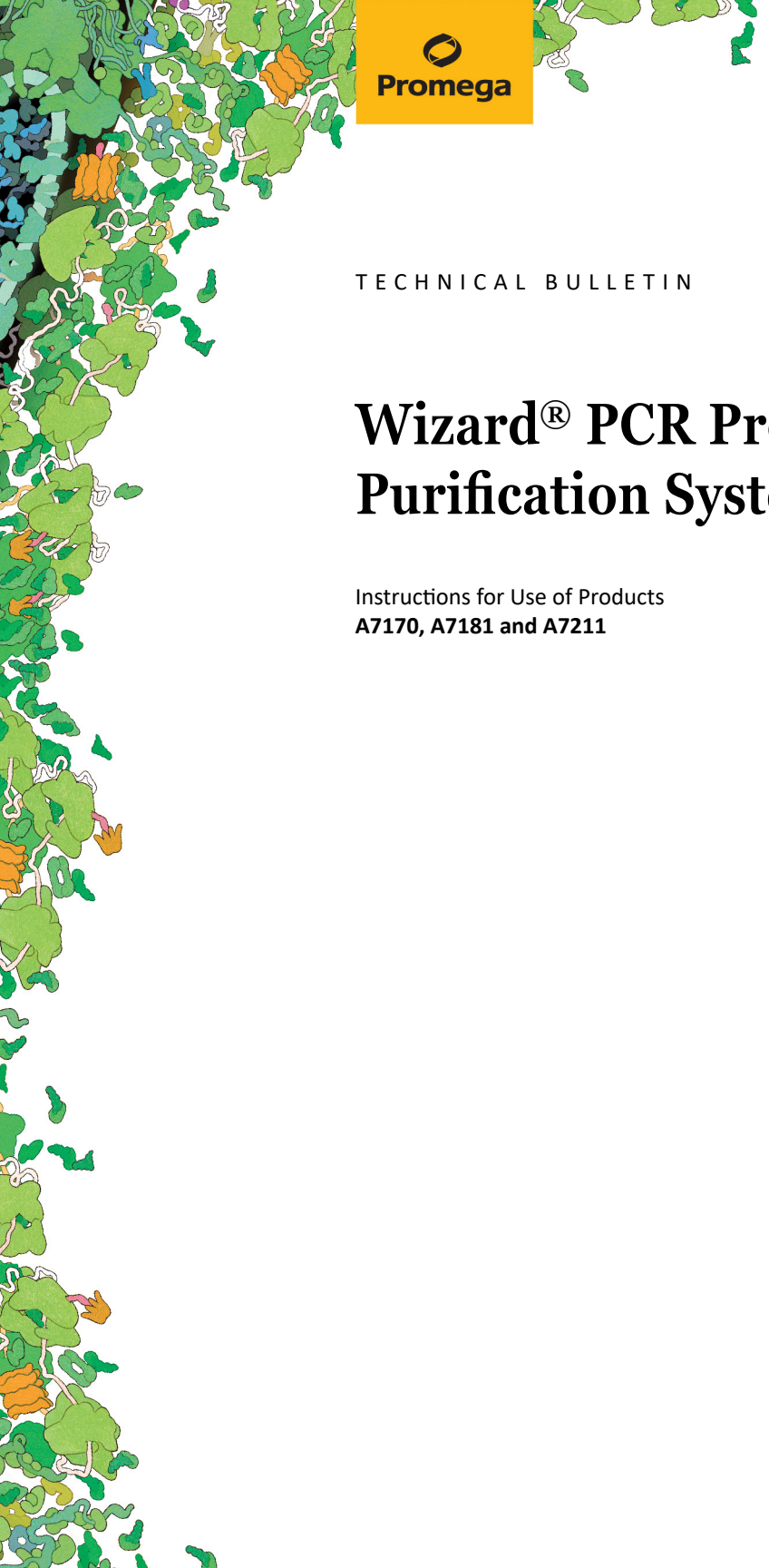

TECHNICAL BULLETIN

Wizard® PCR Preps DNA Purification System

Instructions for Use of Products
A7170, A7181 and A7211

Revised 6/22
TB118

Wizard® PCR Preps DNA Purification System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Wizard® PCR Preps DNA Purification System provides a reliable and inexpensive way to purify double-stranded PCR-amplified DNA. The purification process is quick and simple to perform. Using the 15-minute direct purification method, PCR products are effectively purified from contaminants, including primer-dimers and amplification primers. To separate PCR products from nonspecific amplification products, use either the low-melting temperature agarose method (Section 4.A) or the high-melting-temperature agarose method (Section 4.B). The DNA is eluted from the PCR Preps DNA Purification Resin in water or TE buffer, free of salt or macromolecular contaminants. Multiple PCR Preps may be easily processed at one time with the Vac-Man® (Cat.# A7231) or Vac-Man® Jr. (Cat.# A7660) Laboratory Vacuum Manifold.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Wizard® PCR Preps DNA Purification System	**50 preps**	**A7170**

Each system contains sufficient reagents for 50 isolations of PCR products. Includes:

- 50ml Wizard® PCR Preps DNA Purification Resin
- 5ml Direct Purification Buffer
- 50 Wizard® Minicolumns
- 50 Syringe Barrels (3cc)

PRODUCT	SIZE	CAT. #
Wizard® PCR Preps DNA Purification Resin*	**250ml**	**A7181**
Wizard® Minicolumns	**250 each**	**A7211**

Storage Conditions: Store at room temperature. Protect the resin from exposure to direct sunlight. See the expiration date on the product label.

3. General Considerations

Greater than 95% recovery can be obtained when applying between 50ng and 16µg of a 500bp PCR product to 1ml of resin. Percent recoveries using purification from agarose gel band slices will be lower than percent recoveries using direct purification. Typical yields of 70–90% for 500bp fragments can be expected with purification from low-melting-temperature agarose. DNA yields from high-melting-temperature agarose gel band slices will be low, usually <50% for 500bp fragments. Using the direct purification method (Section 4.C) the percent recoveries shown in the table have been obtained.

DNA Fragment Size	Percent Recovery
3,200bp	≥60%
1,500bp	96%
500bp	98%
300bp	99%
200bp	69%
75bp	3%
50bp	2%

For more efficient recovery of fragments >3kb, increase yields by eluting the DNA with water or TE buffer that has been heated (65–80°C) just prior to elution.

4. Sample Preparation

Four different preparation methods follow. Choose Section 4.A if you are working with low-melting-temperature agarose, Section 4.B if using high-melting-temperature agarose, Section 4.C if purifying DNA directly from PCR or Section 4.D if purifying DNA from a 7M urea polyacrylamide gel.

4.A. Purification of DNA from Low-Melting-Temperature Agarose

Separate PCR products using agarose gel electrophoresis if nonspecific amplification products such as primer dimers must be removed. For high-melting-temperature agarose, see Section 4.B.

1. Separate the PCR products by electrophoresis in a TAE agarose gel containing ethidium bromide using standard protocols (1).

   Do **not** use TBE-containing gels with Wizard® PCR Preps.

2. Excise the desired DNA band using a clean, sterile razor blade or scalpel.

   Note: The band must be visualized with a medium or long wavelength (e.g., ≥300nm) UV light and should be excised quickly to minimize exposure of the DNA to UV light. The band should be isolated in approximately 300µl (300mg) or less of agarose. For isolation of larger agarose slices, see reference 2.

3. Transfer the 300µl (300mg) of agarose slice to a 1.5ml microcentrifuge tube and incubate at 70°C until the agarose is completely melted.

4.A. Purification of DNA from Low-Melting-Temperature Agarose (continued)

Thoroughly mix the PCR Preps resin before removing an aliquot. If crystals or aggregates are present, dissolve them by warming the resin to 25–37°C for 10 minutes. While the resin is insoluble, warming should dissolve aggregates. Cool to 30°C before use.

4. Add 1ml of resin to the melted agarose slice. Mix thoroughly for 20 seconds but do not vortex.
5. If you are using a vacuum manifold, proceed to Section 5.A. Otherwise, proceed to Section 5.B.

4.B. Purification of DNA from High-Melting-Temperature Agarose

Note: Lower yields are obtained using high-melting-temperature agarose compared to using low-melting-temperature agarose. For best results, whenever possible use low-melting-temperature agarose.

1. Separate the PCR products by electrophoresis in a TAE agarose gel containing ethidium bromide using standard protocols (1).

Do **not** use TBE-containing gels with Wizard® PCR Preps.

2. Excise the desired DNA band using a clean, sterile razor blade or scalpel.

   Note: The band must be visualized with a medium or long wavelength (e.g., ≥300nm) UV light and should be excised quickly to minimize exposure of the DNA to UV light. The band should be isolated in approximately 300µl (300mg) or less of agarose. For isolation of larger agarose slices, see reference 2.
3. Transfer the 300µl (300mg) agarose slice to a 1.5ml microcentrifuge or screw cap tube.

Thoroughly mix the PCR Preps resin before removing an aliquot. If crystals or aggregates are present, dissolve them by warming the resin to 25–37°C for 10 minutes. While the resin is insoluble, warming should dissolve aggregates. Cool to 30°C before use.

4. Add 1ml of resin. Incubate at approximately 65°C in a water bath for 5 minutes or until the agarose is completely melted.
5. If you are using a vacuum manifold, proceed to Section 5.A. Otherwise, proceed to Section 5.B.

4.C. Direct Purification of DNA from PCR Amplifications

1. Transfer each completed PCR amplification to a clean microcentrifuge tube.

   Note: If PCR was performed with mineral oil, be mindful to transfer only the aqueous (lower) phase to a clean microcentrifuge tube. The presence of too much mineral oil in the sample can lead to a decreased yield in the PCR product purification.
2. Aliquot 100µl of Direct Purification Buffer into a 12 x 75mm polypropylene tube or a 1.5ml microcentrifuge tube. Add 30–300µl of the PCR product. Vortex briefly to mix.
3. Add 1ml of resin and vortex briefly three times over a 1-minute period.
4. If you are using a vacuum manifold, proceed to Section 5.A. Otherwise, proceed to Section 5.B.

4.D. DNA Isolation from a 7M Urea Polyacrylamide Gel

Moderate percent recoveries can be attained using the following procedure for DNA preparation from a denaturing polyacrylamide gel.

1. Cut the fragment from the denaturing gel and place it in a microcentrifuge tube.
2. Add 100µl of TE buffer to the gel slice. Incubate for at least 30 minutes at 37°C (passive elution).
3. Transfer the 100µl aqueous phase to a new microcentrifuge tube. Add 1ml of resin. Vortex 20 seconds to mix.
4. If you are using a vacuum manifold, proceed to Section 5.A, Otherwise, proceed to Section 5.B.

5. PCR Product Purification

5.A. Purification Using a Vacuum Manifold

Materials to Be Supplied by the User

(Solution composition is provided in Section 6.)

- 80% isopropanol (2-propanol, reagent grade)
- deionized water or TE buffer

Multiple Wizard® PCR Preps (up to 20 samples) can easily be processed simultaneously with the Vac-Man® Laboratory Vacuum Manifold (Cat.# A7231).

! Work quickly when purifying DNA from low-melting-temperature agarose, since cooling of the agarose results in gelling, which will decrease yields.

1. For each PCR product or gel sample, prepare one Wizard® Minicolumn. Attach the provided Syringe Barrel to the Luer-Lok® extension of each Minicolumn. Insert the tip of the Minicolumn/Syringe Barrel assembly into the vacuum manifold.
2. Pipet the resin/DNA mix from Section 4 into the Syringe Barrel. Apply a vacuum to draw the resin/DNA mix into the Minicolumn. Break the vacuum to the Minicolumn.
3. To wash the column, add 2ml of 80% isopropanol to the Syringe Barrel, and reapply a vacuum to draw the solution through the Minicolumn.
4. Dry the resin by continuing to draw a vacuum for 30 seconds after the solution has been pulled through the column. Do not dry the resin for more than 30 seconds. Remove the Syringe Barrel and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge the Minicolumn at 10,000 × *g* in a microcentrifuge for 2 minutes to remove any residual isopropanol.
5. Transfer the Minicolumn to a new microcentrifuge tube. Apply 50µl of water or TE buffer to the Minicolumn and wait 1 minute (the DNA will remain intact on the Minicolumn for up to 30 minutes). Centrifuge the Minicolumn for 20 seconds at 10,000 × *g* to elute the DNA fragment.

! Large DNA fragments require elution with water or TE buffer that has been heated just prior to use. Elute fragments >3kb with water or TE buffer at 65–80°C. Elute fragments >20kb with water or TE buffer at 80°C.

6. Remove and discard the Minicolumn. The purified DNA can be stored in the microcentrifuge tube at 4°C or –20°C.

5.B. Purification Without a Vacuum Manifold

DNA can be purified using a syringe; however, the use of a vacuum source is more reproducible and generally results in higher quality DNA. If small numbers of samples are being processed, we recommend the Vac-Man® Jr. Laboratory Vacuum Manifold. (For a free Vac-Man® Jr. Laboratory Vacuum Manifold, contact Promega.)

Materials to Be Supplied by the User

(Solution composition is provided in Section 6.)

- 80% isopropanol (2-propanol, reagent grade)
- deionized water or TE buffer
- disposable 3ml Luer-Lok® syringes

One disposable 3ml Luer-Lok® syringe is required for each PCR Prep
(e.g., Becton-Dickinson & Co., Cat.# 309657).

Work quickly when purifying DNA from low-melting-temperature agarose because cooling of the agarose results in gelling, which will decrease yields.

1. For each PCR product or gel sample, prepare one Wizard® Minicolumn. Remove and set aside the plunger from a 3ml disposable syringe. Attach the Syringe Barrel to the Luer-Lok® extension of each Minicolumn.
2. Pipet the resin/DNA mix (from Section 4) into the Syringe Barrel. Insert the syringe plunger slowly, and gently push the slurry into the Minicolumn with the syringe plunger.
3. Detach the syringe from the Minicolumn, and remove the plunger from the syringe. Reattach the Syringe Barrel to the Minicolumn. Pipet 2ml of 80% isopropanol into the syringe to wash the column. Insert the syringe plunger into the syringe, and gently push the isopropanol through the Minicolumn.
4. Remove the syringe and transfer the Minicolumn to a 1.5ml microcentrifuge tube. Centrifuge the Minicolumn for 2 minutes at 10,000 × *g* to dry the resin.
5. Transfer the Minicolumn to a new microcentrifuge tube. Apply 50µl of water or TE buffer to the Minicolumn and wait 1 minute (the DNA will remain intact on the Minicolumn for up to 30 minutes). Centrifuge the Minicolumn for 20 seconds at 10,000 × *g* to elute the DNA fragment.

   Large DNA fragments require elution with water or TE buffer that has been heated just prior to use. Elute fragments >3kb with water or TE buffer at 65–80°C. Elute fragments >20kb with water or TE buffer at 80°C.
6. Remove and discard the Minicolumn. The purified DNA can be stored in the microcentrifuge tube at 4°C or –20°C.

6. Composition of Buffers and Solutions

TE Buffer

10mM Tris-HCl (pH 7.5)
1mM EDTA

Direct Purification Buffer

50mM KCl
10mM Tris-HCl (pH 8.8 at 25°C)
1.5mM $MgCl_2$
0.1% Triton® X-100

7. Related Products

DNA Purification

Product	Size	Cat.#
Wizard® SV Gel and PCR Clean-Up System	10 preps	A9280
	50 preps	A9281
	250 preps	A9282
	1,000 preps	A9285
ReliaPrep™ DNA Clean-Up and Concentration System	10 preps	A2891
	50 preps	A2892
	250 preps	A2893
Wizard® DNA Clean-Up System*	100 preps	A7280
Vac-Man® Laboratory Vacuum Manifold	20-sample capacity	A7231
Vac-Man® Jr. Laboratory Vacuum Manifold	2-sample capacity	A7660

*For Laboratory Use.

7. Related Products (continued)

Amplification-Related Products

Product	Size	Cat.#
Access RT-PCR System	500 reactions	A1280
	100 reactions	A1250
Access RT-PCR Introductory System	20 reactions	A1260
GoTaq® PCR Core System I	200 reactions	M7660
GoTaq® PCR Core System II	200 reactions	M7665
GoTaq® G2 DNA Polymerase	100u	M7841
	500u	M7845
	2,500u	M7848
GoTaq® G2 Hot Start Polymerase	100u	M7401
	500u	M7405
	2,500u	M7406
	10,000u	M7408
GoTaq® Long PCR Master Mix	100 reactions	M4021
AMV Reverse Transcriptase	300u	M5101
AMV Reverse Transcriptase, High Concentration	600u	M9004
Recombinant RNasin® Ribonuclease Inhibitor	2,500u	N2511

For Laboratory Use.

Downstream Applications: PCR Cloning

Product	Size	Cat.#
pGEM®-T Vector System I	20 reactions	A3600
pGEM®-T Vector System II (incl. competent cells)	20 reactions	A3610
pGEM®-T Easy Vector System I	20 reactions	A1360
pGEM®-T Easy Vector System II (incl. competent cells)	20 reactions	A1380

For Laboratory Use.

Reagents and dNTPs

Product	Size	Cat.#
Agarose, Low Melting Point, Analytical Grade	25g	V2111
Agarose, LMP, Preparative Grade for Large Fragments (>1,000bp)	25g	V2831
Agarose, LMP, Preparative Grade for Small Fragments (10–1,000bp)	25g	V3841
dATP, 100mM*	40µmol	U1201
dCTP, 100mM*	40µmol	U1221
dGTP, 100mM*	40µmol	U1211
dTTP, 100mM*	40µmol	U1231
dATP, dCTP, dGTP, dTTP, 100mM each*	40µmol/each	U1240
dATP, dCTP, dGTP, dTTP, 100mM each*	10µmol/each	U1330

*For Laboratory Use.

8. References

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Laboratory, Cold Spring Harbor, New York, 6.9.

2. York, C., Birschbach, D. and Navarro, S. (1993) Large-scale gel purification of DNA fragments using Wizard® PCR Preps Resin. *Promega Notes* **43**, 14–7.

9. Summary of Changes

The following changes were made to the 6/22 revision of this document:

1. Removed discontinued products Cat.# A2810 and A7241.
2. Removed selected citations from Section 1.
3. Updated Section 7.
4. Placed document in a new template.