ZYMO RESEARCH

DNA Purification Made Simple

ZymoPURE™ Plasmid Miniprep Kit

Rapid purification of transfection-grade plasmid DNA from up to 5 ml of overnight *E. coli* culture.

Highlights

- Purify up to 100 µg of plasmid DNA in as little as 25 µl directly from a spin-column.
- Purified plasmid DNA contains 50,000 times fewer endotoxins than industry leading minipreps.
- Purify constructs up to ~200 kb in size.

Catalog Numbers:
D4208T, D4209, D4210, D4211, D4212

Scan with your smart-phone camera to view the online protocol/video.

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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Product Contents

ZymoPURE™ Plasmid Miniprep Kit	D4208T (10 prep)	D4209 (50 prep)	D4210 (100 prep)	D4211 (400 prep)	D4212 (800 prep)	Storage Temperature
ZymoPURE™ P1[1] (Red)	3 ml	13 ml	13 ml (2x)	100 ml	210 ml	4°C
ZymoPURE™ P2[2,3] (Green)	3 ml	13 ml	13 ml (2x)	100 ml	210 ml	Room Temp.
ZymoPURE™ P3 (Yellow)	3 ml	13 ml	13 ml (2x)	100 ml	210 ml	Room Temp.
ZymoPURE™ Binding Buffer[3]	3 ml	14 ml	14 ml (2x)	110 ml	110 ml (2x)	Room Temp.
ZymoPURE™ Wash 1	12 ml	20 m (2x)	20 ml (4x)	320 ml	320 ml (2x)	Room Temp.
ZymoPURE™ Wash 2[4]	11 ml	12 ml	23 ml	28 ml (3x)	28 ml (6x)	Room Temp.
ZymoPURE™ Elution Buffer	1 ml	1 ml (2x)	6 ml	12 ml	30 ml	Room Temp.
Zymo-Spin™ II-PX Columns	10 pcs	50 pcs	100 pcs	400 pcs	800 pcs	Room Temp.
Collection Tubes	10 pcs	50 pcs	100 pcs	400 pcs	800 pcs	Room Temp.
Instruction Manual	1 pc	1 pc	1 pc	1 pc	1 pc	Room Temp.

[1] ZymoPURE™ P1 contains RNase A (100 µg/ml) and is stable at room temperature without loss in RNase activity, however, for long-term storage the product should be stored at 4-8° C.
[2] Caution: ZymoPURE™ P2 Buffer contains NaOH. Please use proper safety precautions.
[3] The ZymoPURE™ P2 and ZymoPURE™ Binding Buffer may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!
[4] ZymoPURE™ Wash 2 included with D4208S and D4208T is supplied ready-to-use and does not require the addition of ethanol prior to use. ZymoPURE™ Wash 2 included with D4209, D4210, D4211, and D4212 are supplied as a concentrate and require the addition of ethanol prior to use. See Buffer Preparation (page 4) for instructions.

Specifications

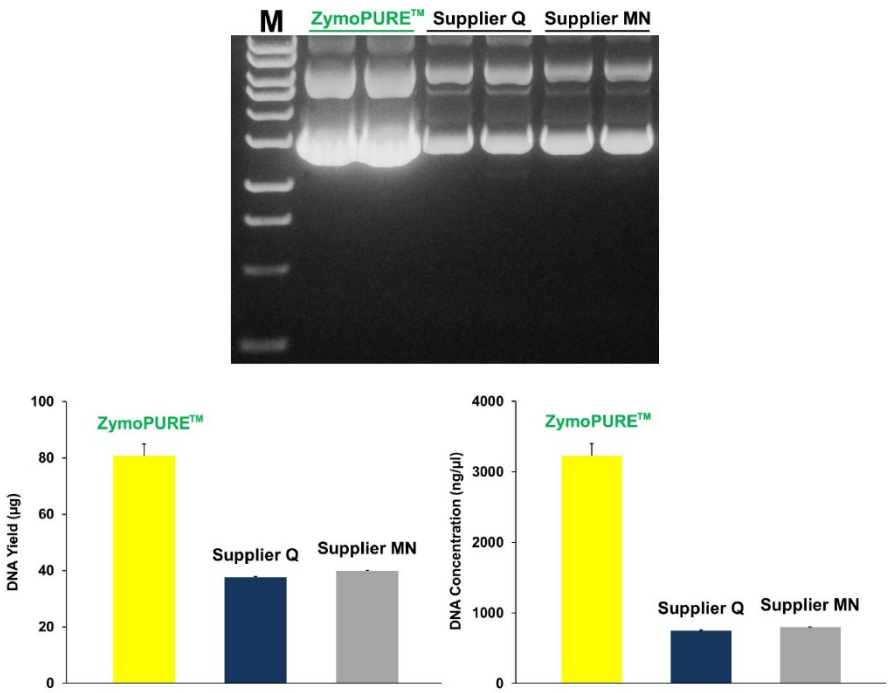
- **DNA Purity** – Eluted DNA is ultrapure, endotoxin-free, and well suited for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, and other sensitive applications.
- **Plasmid DNA Yield** – Up to 100 µg per preparation *(Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of E. coli utilized)*
- **Plasmid DNA Size** – Up to 200 kb
- **Recovery Volume** – ≥ 25 µl of ZymoPURE™ Elution Buffer or DNase free water
- **Processing Time** – 15 min
- **Required Equipment** – Microcentrifuge and/or vacuum manifold (recommended).

Product Description

The **ZymoPURE™ Plasmid Miniprep Kit** features a spin column-based method for the purification of up to 100 µg of ultra-pure endotoxin-free plasmid DNA in less than 15 minutes. The unique spin-column design also provides zero buffer retention and a low elution volume.

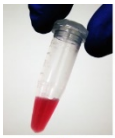
ZymoPURE™ technology uses a modified alkaline lysis method and features novel binding chemistry that yields highly concentrated plasmid DNA (up to 3 µg/µl). In addition, the wash regimen has been optimized to ensure the plasmid DNA is free of endotoxins, salt, protein, and RNA. The result is plasmid DNA suitable for transfection, transformation, lentivirus production, adenovirus production, AAV production, CRISPR, genome editing, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR and other sensitive downstream applications.

As an added convenience, the **ZymoPURE™ Plasmid Miniprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization.



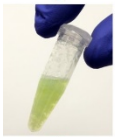
Plasmid DNA yield and concentration from the ZymoPure™ Miniprep Kit compared to other major suppliers. Plasmid DNA (pGL3®) was isolated from 5 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

Procedure Overview



Bacterial cells are resuspended in **ZymoPURE™ P1** (red).

The solution will turn dark purple and viscous following the addition of **ZymoPURE™ P2** (green) indicating bacterial lysis is complete.



The solution will turn yellow and a precipitate will form after adding **ZymoPURE™ P3** (yellow) indicating neutralization is complete.

ZymoPURE™ Binding Buffer is added to the cleared lysate and mixed thoroughly.

The mixture is loaded into the **Zymo-Spin™ II-PX Column** using a vacuum manifold or microcentrifuge.

The **Zymo-Spin™ II-PX Column** is washed using a vacuum manifold or microcentrifuge.

Transfection-grade plasmid DNA is eluted from the **Zymo-Spin™ II-PX Column** using a microcentrifuge.

Protocol

Buffer Preparation:

- ✓ Add 46 ml of 95% ethanol to the **12 ml ZymoPURE™ Wash 2 (Concentrate)** (D4209), 88 ml of 95% ethanol to the **23 ml ZymoPURE™ Wash 2 (Concentrate)** (D4210), or 107 ml of 95% ethanol to the **28 ml ZymoPURE™ Wash 2 (Concentrate)** (D4211 & D4212) before use.

- ✓ The **ZymoPURE™ P2** and **ZymoPURE™ Binding Buffer** may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!

Plasmid DNA Purification

The following procedure should be performed at room temperature (15-30°C).

1. Centrifuge 0.5-5 ml[1] of bacterial culture in a clear 1.5 ml tube at full speed for 15-20 seconds in a microcentrifuge. Discard supernatant.

2. Add 250 µl of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

3. Add 250 µl of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 8-10 times. Do not vortex! Let sit at room temperature for 3 minutes[2]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

4. Add 250 µl of **ZymoPURE™ P3 (Yellow)** and mix thoroughly by inversion. Do not vortex! Invert the tube an additional 5 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form.*

5. Centrifuge the neutralized lysate for 5 minutes at 16,000 *x g*.

6. Transfer exactly 600 µl of supernatant from step 5 into a clean 1.5 ml microcentrifuge tube.

7. Add 260 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 6 and mix thoroughly by vortexing for 15 seconds.

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method.

[1] Depending on the volume of bacterial culture it may be necessary to repeat Step 1 several times.
[2] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA. When processing a large number of samples, work with groups of ≤ 10 at a time.

Vacuum Protocol:

This product is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold[1].

8. Place the **Zymo-Spin™ II-PX Column** onto a vacuum manifold. (If vacuum is not available, see page 7 for the centrifugation protocol.)

9. Add the entire mixture from step 7 into the Zymo-Spin™ II-PX Column. Turn on the vacuum until all of the liquid has passed completely through the column.

10. Add 800 µl of **ZymoPURE™ Wash 1** to the Zymo-Spin™ II-PX Column. Turn on the vacuum until all of the liquid has passed completely through the column.

11. Add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-PX Column. Turn on the vacuum until all of the liquid has passed completely through the column.

12. Add 200 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-PX Column. Turn on the vacuum until all of the liquid has passed completely through the column.

13. Place the **Zymo-Spin™ II-PX Column** in a **Collection Tube** and transfer to a microcentrifuge. Centrifuge at ≥ 10,000 x *g* for 1 minute in order to remove any residual wash buffer.

14. Transfer the **Zymo-Spin™ II-PX Column** into a clean 1.5 ml tube and add 25 µl of **ZymoPURE™ Elution Buffer**[2,3] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

[1] To achieve optimal performance, the vacuum pump should be able to apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove any residual lysate remaining in the matrix.
[2] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
[3] The The DNA yield can be increased by pre-warming the **Zymo PURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.

Centrifugation Protocol:

Perform steps 1-7 as indicated in the general protocol, see page 5.

8. Place a **Zymo-Spin™ II-PX Column** in a Collection Tube.

9. Transfer the entire mixture from step 7 into the Zymo-Spin™ II-PX Column. Incubate the **Zymo-Spin™ II-PX/Collection Tube** assembly at room temperature for 1 minute and then centrifuge at ≥ 10,000 *x g* for 1 min. Discard the flow through[1].

10. Add 800 µl of **ZymoPURE™ Wash 1** to the Zymo-Spin™ II-PX Column and centrifuge at ≥ 10,000 x *g* for 1 min. Discard the flow through.

11. Add 800 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-PX Column and centrifuge at ≥ 10,000 x *g* for 1 min. Discard the flow through.

12. Add 200 µl of **ZymoPURE™ Wash 2** to the Zymo-Spin™ II-PX Column and centrifuge at ≥ 10,000 x *g* for 1 min. Discard the flow through.

13. Centrifuge the Zymo-Spin™ II-PX Column at ≥ 10,000 x *g* for 1 minute in order to remove any residual wash buffer.

14. Transfer the Zymo-Spin™ II-PX Column into a clean 1.5 ml tube and add 25 µl of **ZymoPURE™ Elution Buffer**[2,3] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

[1] The capacity of the collection tube with the column inserted is 900 µl. Empty the collection tube whenever necessary to prevent contamination on the spin-column with the flow-through.
[2] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
[3] The The DNA yield can be increased by pre-warming the **Zymo PURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.

Appendices

Low-Copy Number Protocol

When working with low-copy number plasmid DNA, it is possible to increase plasmid DNA yield by processing up to 10 ml of overnight culture grown in LB using the protocol below. Please be advised that using this protocol will reduce the number of preps that can be performed with this kit because it requires using larger volumes of ZymoPURE P1, P2, P3, and binding buffer.

Plasmid DNA Purification

The following procedure should be performed at room temperature (15-30°C).

1. Centrifuge up to 10 ml[1] of bacterial culture in a clear 2 ml tube at full speed for 15-20 seconds in a microcentrifuge. Discard supernatant.

2. Add 500 µl of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

3. Add 500 µl of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 8-10 times. Do not vortex! Let sit at room temperature for 3 minutes[2]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

4. Add 500 µl of **ZymoPURE™ P3 (Yellow)** and mix thoroughly by inversion. Do not vortex! Invert the tube an additional 5 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form.*

5. Centrifuge the neutralized lysate for 5 minutes at 16,000 *x g*.

6. Transfer exactly 1,200 µl of supernatant from step 5 into a clean 1.5 ml microcentrifuge tube.

7. Add 520 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 6 and mix thoroughly by vortexing for 15 seconds.

   To continue processing the lysate using the recommended vacuum protocol, proceed to page 6. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method. Step 9 in the vacuum and centrifugation protocol must be performed twice because the volume of the lysate and binding buffer mixture is greater than 900 µl.

[1] Depending on the volume of bacterial culture it may be necessary to repeat Step 1 several times.
[2] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA. When processing a large number of samples, work with groups of ≤ 10 at a time.

Gram-Positive Bacteria Protocol

It is possible to isolate plasmid DNA from Gram-Positive species with the ZymoPURE Miniprep Kit. However, the cell walls of the bacteria must be digested with a lytic enzyme prior to alkaline lysis. The protocol below is for Gram-Positive strains that are sensitive to the lytic enzyme Lysozyme.

Plasmid DNA Purification

The following procedure should be performed at room temperature (15-30°C).

1. Centrifuge up to 0.5-5 ml[1] of bacterial culture in a clear 1.5 ml tube at full speed for 15-20 seconds in a microcentrifuge. Discard supernatant.

2. Add 250 µl of **ZymoPURE™ P1 (Red)** containing lysozyme[2] at a final concentration of 1 mg/ml to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

3. Incubate the resuspended cell pellet at 37°C for 15-60 minutes[3].

4. Add 250 µl of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 8-10 times. Do not vortex! Let sit at room temperature for 3 minutes[4]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

5. Add 250 µl of **ZymoPURE™ P3 (Yellow)** and mix thoroughly by inversion. Do not vortex! Invert the tube an additional 5 times after the sample turns completely yellow. *The sample will turn yellow when the neutralization is complete, and a yellowish precipitate will form.*

6. Centrifuge the neutralized lysate for 5 minutes at 16,000 *x g*.

7. Transfer exactly 600 µl of supernatant from step 5 into a clean 1.5 ml microcentrifuge tube.

8. Add 260 µl of **ZymoPURE™ Binding Buffer** to the cleared lysate from step 6 and mix thoroughly by vortexing for 15 seconds.

   To continue processing the lysate using the recommended vacuum protocol, proceed to page 6. If a vacuum is not available, proceed to page 7 for an alternative centrifugation method.

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[1] Depending on the volume of bacterial culture it may be necessary to repeat Step 1 several times.
[2] Lytic enzymes other than lysozyme will require optimization and validation in the ZymoPURE P1 buffer prior to use.
[3] Incubation times will vary depending on the cell density and age of cells. Harvesting the cells at early log phase is recommended for optimal cell wall digestion.
[4] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA. When processing a large number of samples, work with groups of ≤ 10 at a time.

Troubleshooting

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	**Poor aeration of culture.** The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. **The culture was overgrown, undergrown, contaminated, or antibiotics were omitted from the growth medium.** Use a fresh culture for optimal performance. An OD_{600} of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture. **Too much culture used.** Lysis and neutralization will be incomplete resulting in poor lysate clarification. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used. **Incomplete neutralization:** The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface. Make sure the neutralization is complete prior to centrifugation. Invert the tube an additional 3-4 times after the sample turns yellow following the addition of ZymoPURE™ P3. **ZymoPURE P2 and/or ZymoPURE Binding Buffer may have precipitated during shipping.** To completely resuspend the buffers, incubate the bottles at 30-37 ºC for 10 minutes and mix by inversion. DO NOT MICROWAVE. **Less than 600 µl of supernatant was recovered after pelleting the lysate debris.** The ratio of binding buffer to lysate is critical for optimal performance and plasmid DNA yield will be significantly reduced if less than 600 µl of clarified lysate is used.

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	**ZymoPURE Wash 2:** Ensure that the correct volume of ethanol was added to the ZymoPURE™ Wash 2 prior to use. Also, ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol. **Incomplete elution:** For large size plasmids (> 10 kb), add ZymoPURE™ Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution.
Low DNA Quality	**Incomplete neutralization:** Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 3-4 times after the sample turns yellow following the addition of ZymoPURE™ P3. **Insufficient centrifugation:** Make sure that all centrifugation steps are performed at the indicated speed and time. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
RNA in eluate	Ensure that ZymoPURE™ P1 has been stored at 4°C. RNase A can be purchased separately if necessary.
Genomic DNA in eluate	**Improper handling:** Sample was vortexed or handled too roughly. Genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, incomplete lysis or neutralization may contribute to genomic DNA contamination in your eluate. **Overgrown culture.** Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information

Product Description	Catalog No.	Size
ZymoPURE™ Plasmid Miniprep Kit	D4208T D4209 D4210 D4211 D4212	10 Preps. 50 Preps. 100 Preps. 400 Preps. 800 Preps.

Individual Kit Components	Catalog No.	Amount
ZymoPURE™ P1 (Red)	3 ml 13 ml 100 ml 210 ml	D4200-1-3 D4200-1-13 D4200-1-100 D4200-1-210
ZymoPURE™ P2 (Green)	3 ml 13 ml 100 m 210 ml	D4200-2-3 D4200-2-13 D4200-2-100 D4200-2-210
ZymoPURE™ P3 (Yellow)	3 ml 13 ml 100 ml 210 ml	D4200-3-3 D4200-3-13 D4200-3-100 D4200-3-210
ZymoPURE™ Binding Buffer	3 ml 13 ml 100 ml 210 ml	D4200-4-3 D4200-4-13 D4200-4-100 D4200-4-210
ZymoPURE™ Wash 1	20 ml 55 ml 320 ml 420 ml	D4200-5-20 D4200-5-55 D4200-5-320 D4200-5-420
ZymoPURE™ Wash 2 (Concentrate)	10 ml 12 ml 23 ml 28 ml	D4200-6-10 D4200-6-12 D4200-6-23 D4200-6-28
ZymoPURE™ Elution Buffer	6 ml 12 ml 30 ml	D4200-7-6 D4200-7-12 D4200-7-30
Zymo-Spin™ II-PX	50	C1086-50
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

Complete Your Cloning Workflow

✓ **20 Minute Midi & Maxipreps**

ZymoPURE™ Plasmid Prep Kits	Size	Catalog No.
ZymoPURE™ II Plasmid Midiprep Kit	25 Preps. 50 Preps.	D4200 D4201
ZymoPURE™ II Plasmid Maxiprep Kit	10 Preps. 20 Preps.	D4202 D4203
ZymoPURE™ II Plasmid Gigaprep Kit	5 Preps.	D4204

✓ **Simple 20 second High Efficiency Transformations**

Mix & Go! Competent Cells	Size	Catalog No.
DH5α	10 x 100 µl aliquots 96 x 50 µl aliquots 96 x 50 µl aliquots PCR Plate	T3007 T3009 T3010
JM109	10 x 100 µl aliquots 96 x 50 µl aliquots	T3019 T3020
Zymo10B	10 x 100 µl aliquots 96 x 50 µl aliquots	T3003 T3005
HB101	10 x 100 µl aliquots 96 x 50 µl aliquots	T3011 T3013
C600	10 x 100 µl aliquots	T3015
TG1	10 x 100 µl aliquots	T3017

✓ **Recover ultra-pure highly concentrated DNA from PCR & other sources**

DNA Clean & Concentrator™	Size	Catalog No.
DNA Clean & Concentrator™-5	50 Preps. 200 Preps.	D4003 D4004
ZR-96 DNA Clean-Up Kit™	2 x 96 Preps. 4 x 96 Preps.	D4017 D4018
Genomic DNA Clean & Concentrator™-10	25 Preps. 100 Preps.	D4010 D4011

✓ **Rapid extraction of ultra-pure DNA from agarose gels**

Zymoclean Gel DNA Recovery™	Size	Catalog No.
Zymoclean™ Gel DNA Recovery Kit	50 Preps. 200 Preps.	D4001 D4002
Zymoclean™ Large Fragment DNA Recovery Kit	2 x 96 Preps. 4 x 96 Preps.	D4045 D4046

Notes

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Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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