ZYMO RESEARCH

DNA Purification Made Simple™

# ZymoPURE™ II Plasmid Gigaprep Kit

Rapid purification of endotoxin-free plasmid DNA from up to 2.5 L of overnight *E. coli* culture.

## Highlights

- Perform plasmid gigapreps in only 45 minutes using a simple spin-column protocol.
- Purify up to 25 mg of highly concentrated plasmid DNA directly from a spin-column.
- Eluted plasmid DNA is Endotoxin-free and Transfection-Ready.

Catalog Numbers:
D4204

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

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# Product Contents

| ZymoPURE™ II Plasmid Gigaprep Kit | D4204 (5 prep) | Storage Temperature |
|---|---|---|
| ZymoPURE™ P1[1] (Red) | 410 ml (2x) | 4°C |
| ZymoPURE™ P2[2,3] (Blue) | 410 ml (2x) | Room Temp. |
| ZymoPURE™ P3 (Yellow) | 410 ml (2x) | Room Temp. |
| ZymoPURE™ Binding Buffer[3] | 410 ml (2x) | Room Temp. |
| ZymoPURE™ Wash 1 | 55 ml (10x) | Room Temp. |
| ZymoPURE™ Wash 2 (concentrate)[4] | 28 ml (8x) | Room Temp. |
| ZymoPURE™ Elution Buffer | 30 ml | Room Temp. |
| Zymo-Spin™ VI-PX Columns | 5 pcs | Room Temp. |
| 600 ml Reservoir | 5 pcs | Room Temp. |
| ZymoPURE™ Giga Filter | 5 pcs | Room Temp. |
| EndoZero™ III Spin-Column w/15 ml Reservoir-X | 5 pcs | Room Temp. |
| Instruction Manual | 1 pc | - |

[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 D4204 is supplied as a concentrate and require the addition of ethanol prior to use. See Buffer Preparation (page 5) 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, *in vivo* studies, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, and other sensitive applications.
    - Typical Abs260/280 ≥ 1.8 and Abs260/230 ≥ 2.0
    - Endotoxin levels: ≤ 1 EU/µg of plasmid DNA using the Standard Protocol

      ≤ 0.025 EU/µg of plasmid DNA with optional EndoZero™ II Spin-Column
- **Plasmid DNA Yield** – Up to 25 mg per preparation. Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized. Typical yields from 1 Liter of overnight culture grown in LB for high-copy plasmids are 4 – 8 mg.
- **Plasmid DNA Size** – Up to ~200 kb
- **Recovery Volume** – ≥ 3.0 ml of ZymoPURE™ Elution Buffer or DNase-free water
- **Processing Time** – ≤ 45 min
- **Required Equipment** – Vacuum/vacuum manifold and swinging-bucket centrifuge.

# Product Description

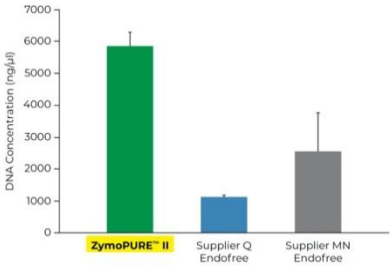
The **ZymoPURE™ II Plasmid Gigaprep Kit** features a simple spin-column based method for the purification of up to 25 mg of transfection grade plasmid DNA in less than 45 minutes. The eluted plasmid DNA is endotoxin-free and ready for immediate use in the most sensitive applications. The unique ZymoPURE™ methodology removes the need for slow gravity flow anion-exchange columns, alcohol precipitation, lengthy endotoxin removal incubations, and time-consuming centrifugation steps.

ZymoPURE™ technology uses a modified alkaline lysis method and features our patented binding chemistry and EZ-Flow™ spin-column design, which enables the highest DNA binding capacity and rapid loading of the lysate and wash buffer, resulting in the fastest purification of highly concentrated (up to 6 mg/ml) plasmid DNA directly from a spin-column. Coupling ZymoPURE™ with the innovative **EndoZero™ III Spin-Columns**, to eliminate residual endotoxins, achieves endotoxin-free plasmid DNA (≤ 0.025 EU/µg of plasmid DNA), making it suitable for transfection, recombinant virus production, lentivirus production, genome editing, *in vivo* studies, sequencing, restriction endonuclease digestion, *in vitro* transcription/translation, PCR, transformation, and other sensitive applications.

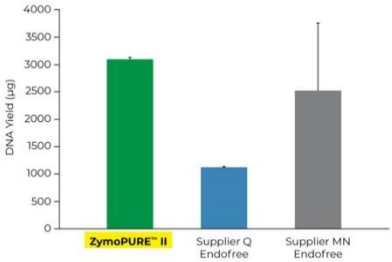
As an added convenience, the **ZymoPURE™ II Plasmid Gigaprep Kit** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization. Bottle top filters are also included for rapid clearing of the lysate.

## Rapid Purification of Highly Concentrated Endotoxin-Free Plasmid DNA


**Up to 6x More Concentrated Plasmid**




**Up to 3x More Plasmid DNA Yield**




**Yield and concentration for plasmid DNA isolated using the ZymoPURE™ II Maxiprep kit compared to two endotoxin-free kits from Supplier Q and Supplier MN.** Plasmid DNA (pGL3®) was isolated from 150 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** (blue) indicating bacterial lysis is complete.

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

The neutralized lysate is loaded into the **ZymoPURE™ Giga Filter** and clarified using a vacuum.

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

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

The **Zymo-Spin™ VI-PX Column** is washed using a vacuum manifold.

Ultra-pure plasmid DNA is eluted from the **Zymo-Spin™ VI-PX Column** using a centrifuge.

The eluted plasmid DNA is passed through the **EndoZero™ III Column** using a centrifuge.

# Protocol

## Buffer Preparation:

- ✓ Add 107 ml of 95% ethanol to the **28 ml ZymoPURE™ Wash 2 (Concentrate)** 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!

## Before Starting:

- ✓ Centrifuge up to 2.5 liters of bacterial culture at ≥ 3,400 *x g* for 20 minutes to pellet the cells[1]. Discard supernatant.

## Plasmid DNA Purification

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

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[2].

1. Add 150 ml of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.

2. Add 150 ml of **ZymoPURE™ P2 (Blue)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes[3]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

3. Add 150 ml of **ZymoPURE™ P3 (Yellow)** and mix gently but 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.*

4. Place the **ZymoPURE™ Giga Filter** onto a 33 mm or 45 mm-neck glass bottle and load the lysate into the ZymoPURE™ Giga Filter. Ensure the ZymoPURE™ Giga Filter is resting securely on top of the glass bottle and wait 10 minutes for the precipitate to float to the top.

5. Connect the ZymoPURE™ Giga Filter to a vacuum source and turn on the vacuum[4] until approximately 375 ml of cleared lysate is recovered. Save the cleared lysate!

   *The volume of lysate recovered from the Giga Filter is critical for optimal plasmid DNA binding to the spin-column. Please refer to page 8 in the appendix regarding the adjustment of the volume of ZymoPURE™ Binding Buffer used in step 6 if the clarified lysate volume is below approximately 375 ml.*

*(continued on next page)*

[1] A vessel with a minimum volume capacity of 500 ml is required to prepare the bacterial lysate.
[2] 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.
[3] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.
[4] Gently pressing down on the top of the **ZymoPURE™ Giga Filter** when the vacuum is applied will guarantee an airtight seal between the filter and neck of the glass bottle.

6. Add 150 ml ZymoPURE™ Binding Buffer to the cleared lysate from step 5 and mix thoroughly by inverting the capped bottle 10 times.

7. Securely attach the **600 ml Reservoir** to the top of the **Zymo-Spin™ VI-PX Column** and place onto a vacuum manifold.

8. With the vacuum off, add the entire mixture from step 6 into the **600 ml Reservoir/Zymo-Spin™ VI-PX Column Assembly**, and then turn on the vacuum until all of the liquid has passed completely through the column.

9. With the vacuum off, add 100 ml of **ZymoPURE™ Wash 1** to the 600 ml Reservoir. Turn on the vacuum until all of the liquid has passed completely through the column.

10. With the vacuum off, add 100 ml of **ZymoPURE™ Wash 2** to the 600 ml Reservoir. Turn on the vacuum until all of the liquid has passed completely through the column. Repeat this wash step.

11. Remove and discard the 600 ml Reservoir and place the Zymo-Spin™ VI-PX Column in a 50 ml conical tube. Centrifuge at ≥ 3,400 x *g* for 10 minutes in a swinging-bucket rotor[1] in order to remove any residual wash buffer.

12. Transfer the column into a clean 50 ml conical tube and add 3 ml of **ZymoPURE™ Elution Buffer**[2,3,4] directly to the column matrix. Wait 5 minutes, and then centrifuge in a swinging-bucket rotor at ≥ 3,400 x *g* for 5 minutes.

13. *Optional:* For removal of residual endotoxins[5,6], ensure the connection between the 15 ml Reservoir-X and EndoZero™ III Spin-Column is finger tight. Place the assembly into a clean 50 ml conical tube and add the entire eluate from Step 12 into the **15 ml Reservoir-X/EndoZero™ III Spin-Column Assembly**, wait 2 minutes, and then centrifuge at 3,400-5,000 *x g* for 10 minutes in a centrifuge. Store the eluted plasmid DNA at ≤ -20°C.

[1] Due to the design of the **Zymo-Spin™ VI-PX Column**, special care needs to be taken when placing the column in the swinging-bucket rotor to ensure there is sufficient clearance between the rotor arms and the top of the column.
[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 DNA yield can be increased by pre-warming the **ZymoPURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.
[4] For plasmid preparations with expected yields of 5 mg or greater, use 5 ml or more of ZymoPURE™ Elution Buffer to elute the plasmid DNA.
[5] This optional step will reduce endotoxin levels from ≤ 1 EU/µg of plasmid DNA to ≤ 0.025 EU/µg of plasmid DNA.
[6] Due to the **EndoZero™ III Spin-Column** chemistry, some plasmid DNA will be lost during this step. The percent of plasmid DNA loss will be dependent on the amount of plasmid DNA that is going through the spin-column. The plasmid DNA loss is generally not significant for moderate and high-copy number plasmids. However, it can be significant for-low copy number plasmids or lower yielding preps.

# Appendices

## Gram-Positive Bacteria Protocol

It is possible to isolate plasmid DNA from Gram-Positive species with the ZymoPURE™ II Gigaprep 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. Add 150 ml of **ZymoPURE™ P1 (Red)** containing lysozyme[1] at a final concentration of 1 mg/ml to the bacterial cell pellet and resuspend completely by vortexing or pipetting. Incubate the resuspended cell pellet at 37°C for 15-60 minutes[2].

2. Add 150 ml of **ZymoPURE™ P2 (Blue)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes[3]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*

3. Add 150 ml of **ZymoPURE™ P3 (Yellow)** and mix gently but 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.*

4. Place the **ZymoPURE™ Giga Filter** onto a 33 mm or 45 mm-neck glass bottle and load the lysate into the ZymoPURE™ Giga Filter. Ensure the ZymoPURE™ Giga Filter is resting securely on top of the glass bottle and wait 10 minutes for the precipitate to float to the top.

5. Connect the ZymoPURE™ Giga Filter to a vacuum source and turn on the vacuum[4] until approximately 375 ml of cleared lysate is recovered. Save the cleared lysate!

   *The volume of lysate recovered from the Giga Filter is critical for optimal plasmid DNA binding to the spin-column. Please refer to page 8 in the appendix regarding the adjustment of the volume of ZymoPURE™ Binding Buffer used in step 6 if the clarified lysate volume is below approximately 375 ml.*

   *To continue processing the lysate, proceed to step 6 on page 6.*

[1] Lytic enzymes other than lysozyme will require optimization and validation in the **ZymoPURE™ P1** buffer prior to use.
[2] Incubation times will vary depending on the culture volume, cell density, and age of cells. Harvesting the cells at early log phase is recommended for optimal cell wall digestion.
[3] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.
[4] Gently pressing down on the top of the **ZymoPURE™ Giga Filter** when the vacuum is applied will guarantee an airtight seal between the filter and neck of the glass bottle.

## Adjusting Volume of Binding Buffer

The ratio of lysate to binding buffer is critical for optimal plasmid DNA binding to the spin-column. Therefore, it is important that approximately 375 ml of cleared lysate is recovered from the ZymoPURE™ Giga Filter during step 5 of the protocol. If the clarified lysate volume is below approximately 375 ml, please adjust the volume of ZymoPURE™ Binding Buffer used in step 6 of the protocol. This can be accomplished by multiplying the volume of recovered lysate by 0.4. Please see the example and table below for reference.

**Example:** For 350 ml of cleared lysate, you will add 140 ml of ZymoPURE™ Binding Buffer to the cleared lysate instead of 150 ml in step 6 of the protocol (350 ml x 0.4 = 140 ml).

Optimal Volume of ZymoPURE™ Binding Buffer for Various Volumes of Lysate

| Approximate Neutralized Lysate Volume | Volume of ZymoPURE Binding Buffer to Add |
|---|---|
| 375 ml | 150 ml |
| 360 ml | 144 ml |
| 350 ml | 140 ml |
| 340 ml | 136 ml |
| 325 ml | 130 ml |
| 300 ml | 120 ml |

# 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.<br><br>**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.<br><br>**Too much culture used.** Lysis and neutralization will be incomplete and the ZymoPURE™ Giga Filter may clog during filtration. 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.<br><br>**Incomplete lysis:** After addition of ZymoPURE™ P2, the solution should change from opaque pink to a clear viscous purple, indicating complete lysis. Different *E. coli* strains often require different growth conditions and may vary in their susceptibility to alkaline lysis.<br><br>**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.<br><br>**ZymoPURE™ P2 and/or ZymoPURE™ Binding Buffer may have precipitated during shipping.** To completely resuspend the buffers, incubate the bottles at 30-37ºC for 10 minutes and mix by inversion. DO NOT MICROWAVE.<br><br>**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. |

| Problem | Possible Causes and Suggested Solutions |
|---|---|
| **Low DNA Yield** | **Less than approximately 375 ml of neutralized lysate was used for the binding step.** The ratio of binding buffer to lysate is critical for optimal plasmid DNA binding to the spin-column. Plasmid DNA yield will be reduced if less than approximately 375 ml is recovered from the giga filter. Please refer to page 8 in the appendix if less then approximately 375 ml of lysate is recovered from the bottle top filter.<br><br>**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 5 times after the sample turns yellow following the addition of ZymoPURE™ P3.<br><br>**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.<br><br>**Overgrown culture.** Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures. |

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| **ZymoPURE™ II Plasmid Gigaprep Kit** | D4204 | 5 Preps. |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **ZymoPURE™ P1 (Red)** | D4200-1-150<br>D4200-1-210<br>D4200-1-410 | 150 ml<br>210 ml<br>410 ml |
| **ZymoPURE™ P2 (Green)** | D4200-2-150<br>D4200-2-210<br>D4200-2-410 | 150 ml<br>210 ml<br>410 ml |
| **ZymoPURE™ P3 (Yellow)** | D4200-3-150<br>D4200-3-210<br>D4200-3-410 | 150 ml<br>210 ml<br>410 ml |
| **ZymoPURE™ Binding Buffer** | D4200-4-150<br>D4200-4-210<br>D4200-4-410 | 150 ml<br>210 ml<br>410 ml |
| **ZymoPURE™ Wash 1** | D4200-5-55 | 55 ml |
| **ZymoPURE™ Wash 2 (Concentrate)** | D4200-6-28 | 28 ml |
| **ZymoPURE™ Elution Buffer** | D4200-7-6<br>D4200-7-12<br>D4200-7-30 | 6 ml<br>12 ml<br>30 ml |
| **Zymo-Spin™ VI-PX Column** | C1080-5 | 5 |
| **600 ml Reservoir** | C1033-5 | 5 |
| **ZymoPURE™ Giga Filter** | C1038-1 | 1 |
| **EndoZero™ III Spin-Column w/15 ml Reservoir-X** | C1096-5 | 10 |

# Complete Your Cloning Workflow

✓ **Transfection-grade plasmid DNA from a miniprep**

| ZymoPURE™ Plasmid Miniprep | Size | Catalog No. |
|---|---|---|
| **ZymoPURE™ Plasmid Miniprep Kit** | 10 Preps.<br>50 Preps.<br>100 Preps.<br>400 Preps.<br>800 Preps. | D4208T<br>D4309<br>D4210<br>D4211<br>D4212 |

✓ **16 Minute Endotoxin-Free Midi & Maxipreps**

| ZymoPURE™ II Plasmid Prep Kits | Size | Catalog No. |
|---|---|---|
| **ZymoPURE™ II Plasmid Midiprep Kit** | 25 Preps.<br>50 Preps. | D4200<br>D4201 |
| **ZymoPURE™ II Plasmid Maxiprep Kit** | 10 Preps.<br>20 Preps. | D4202<br>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<br>96 x 50 µl aliquots<br>96 x 50 µl aliquots PCR Plate | T3007<br>T3009<br>T3010 |
| **JM109** | 10 x 100 µl aliquots<br>96 x 50 µl aliquots | T3019<br>T3020 |
| **Zymo10B** | 10 x 100 µl aliquots<br>96 x 50 µl aliquots | T3003<br>T3005 |
| **HB101** | 10 x 100 µl aliquots<br>96 x 50 µl aliquots | T3011<br>T3013 |
| **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.<br>200 Preps. | D4003<br>D4004 |
| **ZR-96 DNA Clean-Up Kit™** | 2 x 96 Preps.<br>4 x 96 Preps. | D4017<br>D4018 |

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

| Zymoclean Gel DNA Recovery™ | Size | Catalog No. |
|---|---|---|
| **Zymoclean™ Gel DNA Recovery Kit** | 50 Preps.<br>200 Preps. | D4001<br>D4002 |

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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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