



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zyppy™ Plasmid Maxiprep Kit

Catalog Nos. **D4027 & D4028** (Patent Pending)

Highlights

- Easy and versatile procedure: lyse cells then centrifuge or vacuum, wash, and elute DNA.
- The fastest, simplest procedure for purifying the highest quality endonuclease-free plasmid DNA.
- Innovative colored buffers* permit error-free visual identification of complete bacterial cell lysis and neutralization.

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Product Contents:

Zyppy™ Plasmid Maxiprep Kit (Kit Size)	D4027 (10 preps.)	D4028 (20 preps.)	Storage Temperature
P1 Buffer¹ (Red)	160 ml	320 ml	4 - 8°C after adding RNase A.
P2 Buffer*² (Green)	160 ml	320 ml	Room Temp.
P3 Buffer* (Yellow)	220 ml	440 ml	Room Temp.
Endo-Wash Buffer²	120 ml	240 ml	Room Temp.
Zyppy™ Wash Buffer (concentrate)³	24 ml	48 ml	Room Temp.
Zyppy™ Elution Buffer	30 ml	60 ml	Room Temp.
RNase A	8 mg	2 x 8 mg	Room Temp.
Zymo-Spin™ VI Columns⁴	10	20	Room Temp.
Zymo-Maxi Filter™ Columns⁴	10	20	Room Temp.
Instruction Manual	1	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

¹ Add the RNase A to P1 Buffer and store at 4-8°C. The final concentration of RNase A will be 50 µg/mL.

² P2 Buffer and Endo-Wash Buffer may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

³ Add 96 ml of 100% ethanol or 104 ml of 95% ethanol to the 24 ml Zyppy Wash Buffer concentrate (D4019), or add 192 ml of 100% ethanol or 208 ml of 95% ethanol to the 48 ml Zyppy Wash Buffer concentrate (D4020) before use.

⁴ The Zymo-Spin™ VI and Zymo-Maxi Filter™ columns are pre-assembled as a single unit and are ready for use with the recommended protocol (page 4). For the alternative protocol (page 5), only the Zymo-Spin™ VI column is needed and should be separated from the Zymo-Spin™ VI/Zymo-Maxi Filter™ column assembly prior to use.

*Caution: P2 Buffer contains NaOH and Buffer P3 Contains chaotropic reagents. Please use proper safety precautions with these reagents.

Note: Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product, please call 1-888-882-9682.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. 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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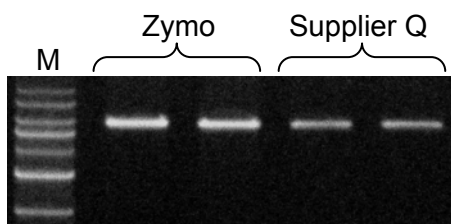
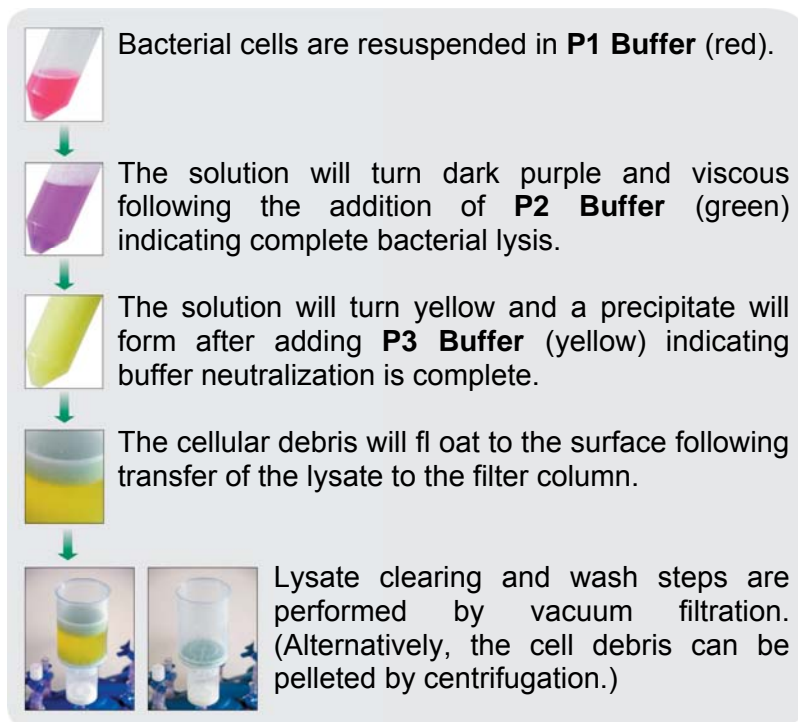
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Product Description

The **Zyppy™ Plasmid Maxiprep Kit** employs a modified alkaline lysis method in conjunction with spin-column purification to isolate high quality *endonuclease-free* plasmid DNA in minutes. The purified DNA is suitable for use in restriction endonuclease digestion, ligation, bacterial transformation, PCR amplification, DNA sequencing and other sensitive downstream applications.

The innovative colored buffers included in the kit permits error-free visualization identification of complete bacterial cell lysis and neutralization. Additionally, the uniquely designed **Zymo-Maxi Filter™** column permits lysate clearing without centrifugation while the high capacity DNA-binding **Zymo-Spin™ VI** column allows for low 2–3 ml elution volumes, eliminating the eluted plasmid DNA precipitation and resuspension steps common to other column-based maxiprep procedures.

The **Zyppy™ Plasmid Maxiprep Kit** is designed for use with a centrifuge, a vacuum manifold, or a combination of both procedures and provides the greatest flexibility in large scale plasmid DNA purification from *E. coli*. An overview of the purification procedure is shown below.



*Bam*HI digestion of plasmid DNA (pUC18) isolated from a 150 ml *E. coli* culture using the **Zyppy™ Plasmid Maxiprep Kit** or a kit from Supplier Q. Performed in duplicate. M, ZR 1 kb DNA Marker.

For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Specifications:

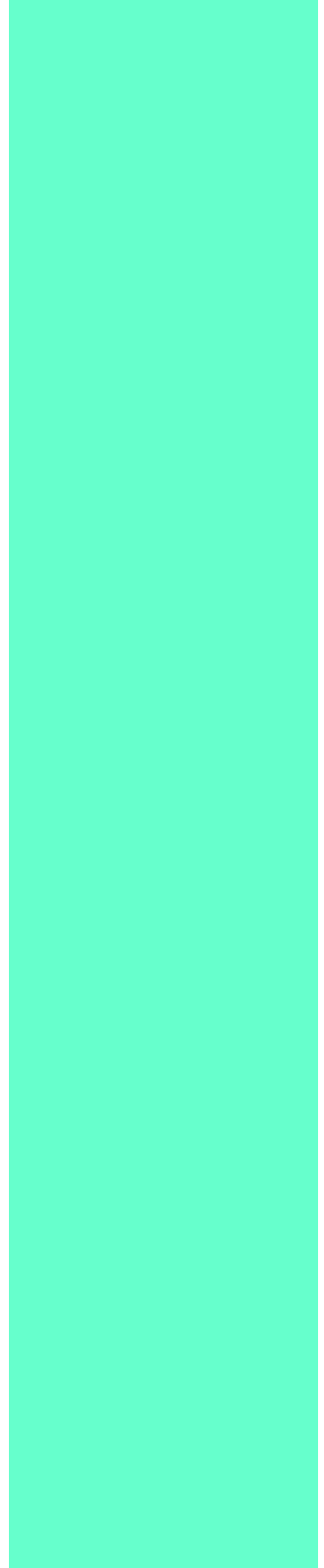
- **DNA Purity:** Plasmid DNA is well suited for ligation, sequencing, restriction endonuclease digestion, *in vitro* transcription, and other sensitive applications requiring pure DNA. Typical Abs_{260/280} index is ≥ 1.8.
- **Plasmid DNA Yield:** ≥ 500 µg per preparation, depending on the plasmid copy number, culture growth conditions, and strain of *E. coli* utilized.
- **Plasmid DNA Size:** Up to 25 kb.
- **Recovery Volume:** ≥ 2 ml.
- **Procedure:** Performed at room temperature (15-30°C) using a centrifuge and/or a vacuum manifold.

Buffer Preparation:

1. Add the **RNase A** to **P1 Buffer**: aliquot one ml of **P1 Buffer** into the tube containing the lyophilized **RNase A**, mix, and transfer the solution back into the **P1 Buffer** bottle. The final concentration of **RNase A** will be 50 µg/ml. Store at 4-8 °C.
2. Add 96 ml of 100% ethanol or 104 ml of 95% ethanol to the 24 ml **Zyppy™ Wash Buffer** concentrate (D4027), or add 192 ml of 100% ethanol or 208 ml of 95% ethanol to the 48 ml **Zyppy™ Wash Buffer** concentrate (D4028) before use.
3. **P2 Buffer** and **Endo-Wash Buffer** may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10-20 minutes and mix by inversion. **DO NOT MICROWAVE.**

Vacuum Pump and Manifold:

- The vacuum pump should be a single- or double-staged unit capable of producing approximately 650 mm Hg pressure at the vacuum manifold.
- This product is compatible with any conventional vacuum-based manifold.



Recommended Protocol:

Isolation of plasmid DNA using a vacuum manifold

The following procedure is performed at room temperature.

P2 Buffer and Endo-Wash Buffer may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30-37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

1. Centrifuge up to 150 ml of fresh bacterial culture at $\geq 3,400 \times g$ for 10 minutes. Discard supernatant.
2. Add 15 ml of **P1 Buffer (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
3. Add 15 ml of **P2 Buffer (Green)** and mix *immediately* by inverting the tube 4-6 times. Let stand for one minute to lyse the cells completely.
4. Add 20 ml of **P3 Buffer (Yellow)** and mix gently but thoroughly. Incubate on ice for 5 minutes. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*
5. Place the **Zymo-Maxi Filter™/Zymo-Spin™ VI** column assembly onto a vacuum manifold.
6. Add the entire mixture into the blue **Zymo-Maxi Filter™** column, let the cell debris float to the surface and turn on the vacuum until all of the liquid has passed completely through both columns.
7. Remove and discard the blue **Zymo-Maxi Filter™** column from the top of the **Zymo-Spin™ VI** column.
8. Add 10 ml of **Endo-Wash Buffer** to the **Zymo-Spin™ VI** column and turn on the vacuum.¹
9. After the **Endo-Wash Buffer** has completely passed through the **Zymo-Spin™ VI** column, add 10 ml of **Zyppy™ Wash Buffer**² and turn on the vacuum. Leave the vacuum on for an additional 5 minutes to remove all residual **Zyppy™ Wash Buffer**.
10. Transfer the **Zymo-Spin™ VI** column into a clean 50 ml conical tube then add 2-3 ml of **Zyppy™ Elution Buffer**^{3, 4} to the column. Incubate at RT for one minute, then centrifuge at $\geq 3,400 \times g$ for 1 minute to elute the plasmid DNA.

Notes:

¹ Alternatively, the two wash steps can be performed in a large centrifuge by placing the **Zymo-Spin™ VI** column into a new 50 ml conical tube. Wash using 10 ml of each buffer and centrifuge at $\geq 3,400 \times g$ for 5 minutes respectively. Empty the conical tube after each wash to prevent contamination of the spin column.

² Ensure that ethanol has been added to the concentrated **Zyppy™ Wash Buffer** before use

³ The **Zyppy™ Elution Buffer** contains 0.1 mM EDTA. If required, pure water can also be used to elute the DNA

⁴ The DNA yield can be increased by incubating the **Zymo-Spin™ VI** column at 25 to 50 °C for 1 to 3 minutes prior to centrifugation.

Alternate Protocol:

Isolation of plasmid DNA using a centrifuge

The following procedure is performed at room temperature.

P2 Buffer and Endo-Wash Buffer may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30-37 °C for 10-20 minutes and mix by inversion. DO NOT MICROWAVE.

1. Centrifuge up to 150 ml of fresh bacterial culture at $\geq 3,400 \times g$ for 10 minutes. Discard supernatant.
2. Add 15 ml of cold **P1 Buffer (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
3. Add 15 ml of **P2 Buffer (Green)** and mix *immediately* by inverting the tube 4-6 times. Let stand for one minute to lyse the cells completely.
4. Add 20 ml of **P3 Buffer (Yellow)** and mix gently but thoroughly. Incubate on ice for 5 minutes. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*
5. Transfer the entire mixture into a 50 ml conical tube. Centrifuge at $\geq 3,400 \times g$ for 10 min to pellet the bacterial cell debris (During the centrifugation, separate the **Zymo-Maxi Filter™/Zymo-Spin™ VI** column assembly and place the smaller **Zymo-Spin™ VI** column into a new 50 ml conical tube).
6. Add 10 ml of the supernatant from step 5 into the **Zymo-Spin™ VI** column, place the cap on the conical tube, and centrifuge at $\geq 3,400 \times g$ for two minutes. Discard the flow-through. *Repeat this step until all of the supernatant from step 5 has been filtered through the **Zymo-Spin™ VI** column.*¹
7. Add 10 ml of **Endo-Wash Buffer** to the **Zymo-Spin™ VI** column and centrifuge at $\geq 3,400 \times g$ for 30 seconds. Discard the flow-through.¹
8. Add 10 ml of **Zyppy™ Wash Buffer**² to the **Zymo-Spin™ VI** column and centrifuge at $\geq 3,400 \times g$ for one minute. Discard the flow-through.
9. Transfer the **Zymo-Spin™ VI** column into a clean 50 ml conical tube then add 2-3 ml of **Zyppy™ Elution Buffer**^{3, 4} to the column. Incubate at RT for one minute, then centrifuge at $\geq 3,400 \times g$ for one minute to elute the plasmid DNA.

Notes:

¹ The maximum capacity of the conical tube with the **Zymo-Spin™ VI** column inserted is 12 ml. Empty the conical tube whenever necessary to prevent contamination of the spin column.

² Ensure that ethanol has been added to the concentrated **Zyppy™ Wash Buffer** before use

³ The **Zyppy™ Elution Buffer** contains 0.1 mM EDTA. If required, pure water can also be used to elute the DNA

⁴ The DNA yield can be increased by incubating the **Zymo-Spin™ VI** column at 25 to 50 °C for 1 to 3 minutes prior to centrifugation.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
<i>Culture growth conditions</i>	<ul style="list-style-type: none"> • Poor aeration of culture. The optimal culture volume to air volume ratio is 1:15 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 or contaminated. Use a fresh culture for optimal performance. • Antibiotics were omitted from the growth medium.
<i>Procedural errors</i>	<ul style="list-style-type: none"> • Incomplete lysis: After addition of P2 Buffer, the solution should change from opaque pink to clear, viscous purple, indicating complete lysis. Different <i>E. coli</i> strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. • Incomplete neutralization: Cell debris will float to the surface after centrifugation and the pellet may appear “puffy”. Make sure the neutralization is complete prior to centrifugation. Invert the tube an additional 2 – 3 times after the sample turns yellow following the addition of P3 Buffer. • Too much culture used. Lysis and Neutralization will be incomplete and the Zymo-Maxi Filter™ column may clog during vacuum filtration. • Insufficient centrifugation: make sure that all centrifugation steps are performed $\geq 3,400 \times g$. If a lower centrifuge speed is used, then extend the centrifugation time to compensate.
<i>P2 Buffer and Endo-Wash Buffer precipitation</i>	<ul style="list-style-type: none"> • Both buffers may have precipitated during shipping. To completely resuspend the buffers, incubate the bottles at 30 – 37 °C for 10 – 20 minutes and mix by inversion. DO NOT MICROWAVE.
<i>Wash buffer</i>	<ul style="list-style-type: none"> • Ensure that ethanol has been added to the wash buffer.
<i>DNA elution</i>	<ul style="list-style-type: none"> • Incomplete elution: For large size plasmids (> 10 kb), add Zypzy™ Elution Buffer and incubate the column for 5 – 10 minutes before centrifugation. Also, pre-warm the Zypzy™ Elution Buffer to 50 °C prior to elution. • Ethanol contamination in eluate. Vacuum or centrifuge the Zymo-Spin™ VI column matrix to dryness as indicated in the protocol prior to adding the Zypzy™ Elution Buffer.
Low DNA Quality	
<i>DNA does not perform well</i>	<ul style="list-style-type: none"> • Incomplete neutralization: Incomplete neutralization generates poor quality supernatant and results in loading too much cell debris onto the column. Ensure that neutralization is complete by inverting the sample an additional 2 – 3 times after the addition of P3 Buffer. • Centrifuge procedure: The Zymo-Spin™ VI column tip is contaminated with wash buffer flowthrough. Empty the collection tube when recommended in the protocol.
<i>RNA in eluate</i>	<ul style="list-style-type: none"> • Ensure that RNase A has been added to the P1 Buffer (store at 4 – 8 °C).
<i>Genomic DNA in eluate</i>	<ul style="list-style-type: none"> • 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, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample. • Overgrown culture. Older cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information:

Product Description	Catalog No.	Kit Size
Zyppy™ Plasmid Maxiprep Kit	D4027	10 preps.
Zyppy™ Plasmid Maxiprep Kit	D4028	20 preps.

For Individual Sale	Catalog No.	Amount
P1 Buffer (Red)	D4027-1-160	160 ml
	D4027-1-320	320 ml
P2 Buffer (Green)	D4027-2-160	160 ml
	D4027-2-320	320 ml
P3 Buffer (Yellow)	D4027-3-220	200 ml
	D4027-3-440	440 ml
Endo-Wash Buffer	D4036-3-120	120 ml
	D4036-3-240	240 ml
Zyppy™ Wash Buffer (concentrate)	D4036-4-24	24 ml
	D4036-4-48	48 ml
Zyppy™ Elution Buffer	D4036-5-30	30 ml
	D4036-5-60	60 ml
RNase A	E1008-2	2 mg
	E1008-8	8 mg
Zymo-Spin™ VI Columns	C1013-10	10
Zymo-Maxi Filter™ Columns	C1017-10	10

Popular DNA Purification & Analysis Products from Zymo

Product	Description	Kit Size (Preps)	Catalog No. (Column Format)
DNA Clean & Concentrator-5™	Clean & concentrate DNA from any reaction or “crude” preparation in 2 minutes. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50	D4003 (uncapped)
		200	D4004 (uncapped)
		50	D4013 (capped)
		200	D4014 (capped)
DNA Clean & Concentrator-25™	Clean & concentrate DNA in minutes. 25 µl minimum elution volume allows for highly concentrated DNA. Designed for purifying up to 25 µg of DNA.	50	D4005
		200	D4006
DNA Clean & Concentrator-100™	Clean & concentrate DNA in minutes. 100 µl minimum elution volume allows for highly concentrated DNA. Designed for purifying up to 100 µg of DNA.	25	D4029
		50	D4030
DNA Clean & Concentrator-500™	Clean & concentrate DNA in minutes. 1 ml minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 500 µg of DNA.	10	D4031
		20	D4032
ZR-96 DNA Clean & Concentrator-5™	Quick (15 minute), high-output recovery of pure DNA from PCR, endonuclease digestions, plasmid preparations, etc. 10-15 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	2x96	D4023
		4x96	D4024
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes	50	D4001
		200	D4002
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2x96	D4021
		4x96	D4022
Pinpoint Slide DNA Isolation System™	Recover genomic DNA from paraffin-embedded or fresh tissue sections for PCR. Ideal for isolating DNA from clinical tissue samples.	50	D3001
Zyppy™ Plasmid Miniprep Kit	Pellet-Free™ plasmid DNA purification in minutes: (alkaline lysis/spin column format for low 30 µl elution volume).	50	D4036
		100	D4019
		400	D4020
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in minutes: (alkaline lysis/spin column format and 150 µl minimum elution volume).	25	D4025
		50	D4026
Zyppy™ Plasmid Maxiprep Kit	High-purity plasmid DNA purification in minutes: (alkaline lysis/spin column format and 2 ml minimum elution volume).	10	D4027
		20	D4028
ZR Genomic DNA I Kit™	Genomic DNA isolation from whole blood, tissue culture cells, solid tissue and liquid samples. (Silica bead format is scalable to fit your requirements).	100	D3004
		400	D3005
ZR Genomic DNA II Kit™	Genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	50	D3006 (uncapped)
		200	D3007 (uncapped)
		50	D3024 (capped)
		200	D3025 (capped)
ZR-96 Genomic DNA Kit™	High-output genomic DNA purification from whole blood, tissue culture cells, solid tissue and liquid samples. No requirement for beads or phenol chloroform.	2x96	D3010
		4x96	D3011
ZR Soil microbe DNA Kit™	Simple, rapid isolation of humic-free, PCR-quality genomic DNA from soil microbes.	50	D6001
ZR Fungal/Bacterial DNA Kit™	Simple, rapid isolation of PCR-quality genomic DNA from fungi.	50	D6005
ZR Fecal DNA Kit™	Simple, rapid isolation of PCR-quality genomic DNA from feces.	50	D6010
ZR Viral DNA Kit™	Isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10 ⁵ cells per ml.	50	D3015
		200	D3016
ZR-96 Viral DNA Kit™	High-output (96-well) isolation of viral DNA from cell-free body fluids or sample mixtures containing cells at concentrations less than 10 ⁵ cells per ml.	2x96	D3017
		4x96	D3018
EZ DNA Methylation Kit™	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via the <u>chemical-denaturation</u> of DNA using our specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using <i>Fast-Spin</i> column technology. Ultra-pure recovered DNA can be used for PCR and bisulfite sequencing applications.	50	D5001
		200	D5002
		2x96	D5003
EZ DNA Methylation-Gold Kit™	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via <u>heat-denaturation</u> of DNA using our specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using <i>Fast-Spin</i> column technology. Ultra-pure recovered DNA can be used for PCR and bisulfite sequencing applications. <i>3 hour processing time!</i>	50	D5005
		200	D5006
		2x96	D5007

*Bulk quantities are available upon request. Please contact: busdev@zymoresearch.com or call 1-888-882-9682 for assistance.

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