

INSTRUCTION MANUAL

ZR-96 DNA Clean & Concentrator™-5 Catalog Nos. **D4023 & D4024**

Highlights

- Quick, high throughput recovery of ultra-pure DNA from PCR, enzymatic reactions, and other sources.
- DNA can be eluted in as little as 10 µl and is ideal for DNA ligation, sequencing, labeling, PCR, microarray, transfection, transformation, restriction digestion, etc.

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

Product Contents

| ZR-96 DNA Clean & Concentrator™-5 (Kit Size) | D4023 (2 x 96 Preps.) | D4024 (4 x 96 Preps.) | Storage Temperature |
|--|------------------------------------|------------------------------------|--------------------------------|
| DNA Binding Buffer | 100 ml | 2 x100 ml | Room Temp. |
| DNA Wash Buffer* | 24 ml | 48 ml | Room Temp. |
| DNA Elution Buffer | 10 ml | 16 ml | Room Temp. |
| Zymo-Spin™ I-96 Plate | 2 | 4 | Room Temp. |
| Collection Plate | 2 | 4 | Room Temp. |
| Elution Plate | 2 | 4 | Room Temp. |
| Instruction Manual | 1 | 1 | - |

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 the highest performance and reliability.

¹ Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label.

Specifications

- **DNA Purity** – High-quality DNA ($A_{260}/A_{280} >1.8$) ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA per well can be eluted into as little as 10 µl of low salt **DNA Elution Buffer** or water. For DNA 50 bp to 10 kb, the recovery is 70-90%. For DNA 11 kb to 23 kb, the recovery is 50-70%.
- **Sample Sources** – DNA from enzymatic reactions (e.g., PCR, restriction endonuclease digestions), plasmid preparations, and impure preparations.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

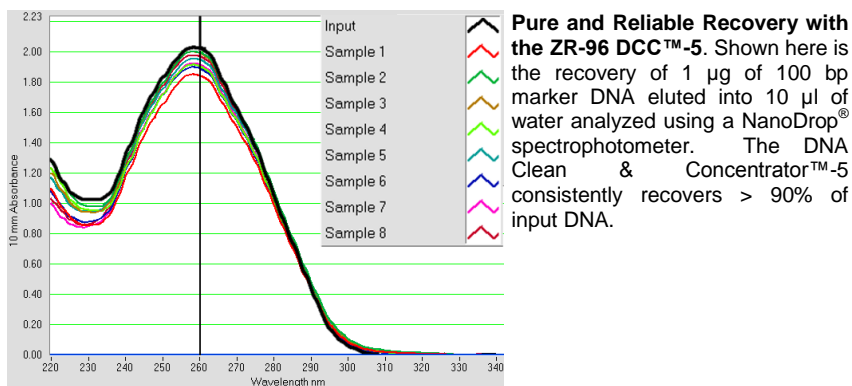
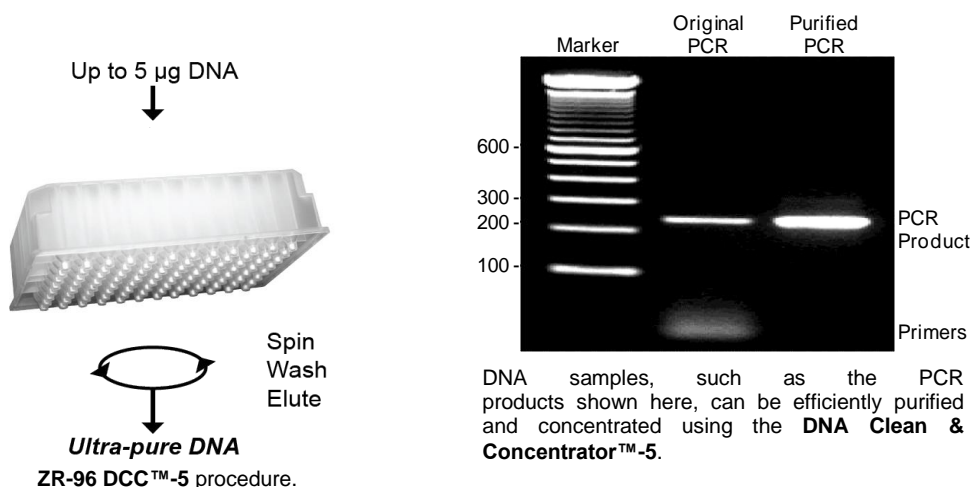
Note: ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility. NanoDrop® is a registered trademark of NanoDrop Technologies, Inc.

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Product Description

The **ZR-96 DNA Clean & Concentrator™-5 (ZR-96 DCC™-5)** provides a hassle-free method for the rapid, high throughput purification and concentration of high-quality DNA from PCR, endonuclease digestions, cell lysates and other impure DNA preparations. It can also be used for post-RT cDNA clean-up and purification of sequencing-ready DNA from M13 phage. Simply add the specially formulated **DNA Binding Buffer** to your sample and transfer to the wells of the supplied **Zymo-Spin™ I-96 Plate**. There is no need for organic denaturants or chloroform. Instead, the product features *Fast-Spin* technology to yield DNA that is free of salts and contaminants in just minutes. DNA purified using the **ZR-96 DCC™-5** is suitable for nucleotide sequencing, microarray analysis, PCR, and restriction endonuclease digestion procedures.



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Available Formats

| | DCC™-5 | DCC™-25 | DCC™-100 | DCC™-500 | Genomic DCC™ | ZR-96 DCC™-5 |
|--------------|---|---|---|---|---|---|
| |  |  |  |  |  |  |
| Name | Zymo-Spin™ I & IC | Zymo-Spin™ II & IIC | Zymo-Spin™ V | Zymo-Spin™ VI | Zymo-Spin™ IC-XL | Zymo-Spin™ I-96 |
| Capacity | 5 µg/ prep. | 25 µg/ prep. | 100 µg/ prep. | 500 µg/ prep. | 10 µg/ prep. | 5 µg/ well. |
| Elution Vol. | ≥ 6 µl | ≥ 25 µl | ≥ 150 µl | ≥ 2 ml | ≥ 10 µl | ≥ 10 µl |
| Cat. Nos. | D4003, D4013 | D4005, D4033 | D4029, D4030 | D4031, D4032 | D4010, D4011 | D4023, D4024 |

Typical DCC™ Applications

| | |
|--|---|
| Post-PCR DNA Clean-up | Efficient desalting of DNA with the removal of DNA polymerases, primers and free dNTPs. |
| DNA Clean-up From Enzymatic Reactions | Efficient desalting of DNA with the removal of DNA polymerases, modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, etc. |
| Post-Reverse Transcription (RT) & cDNA Clean-up | Efficiently purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template. |
| Plasmid DNA Clean-up | Efficiently purifies plasmid DNA from "home-made" preparations of cell free lysates or from commercial kits. Plasmid DNA purified and concentrated using the DCC™ has proven an excellent substrate for high quality DNA sequencing. |
| Isotope and Dye Removal | Efficiently removes unincorporated fluorescent (<i>i.e.</i> , AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, etc.) and radiolabeled dNTP derivatives from DNA following <i>in vitro</i> labeling reactions. |
| Purification of M13 ssDNA | The DCC™ can be used for the rapid isolation of single stranded M13 phage DNA directly from phage-infected <i>E. coli</i> culture supernatant. |

- ✓ For purification of short DNA or RNA oligonucleotides ≥16 nt, use the **Oligo Clean & Concentrator (D4060, D4061)**.
- ✓ For ChIP (Chromatin Immunoprecipitation) sample cleanup, use the **ChIP DNA Clean & Concentrator (D5201, D5205)** for high quality DNA from any step in a standard ChIP protocol.
- ✓ For post-cycle sequencing samples, use the **ZR Sequencing DNA Clean-up Kit (D4050, D4051)** for dye blob elimination.
- ✓ For samples containing PCR inhibitors, use the **OneStep™ PCR Inhibitor Removal Kit (D6030, D6035)**.

Selected Citations

- Li, N. (2010). Whole genome DNA methylation analysis based on high throughput sequencing technology. *Methods*, 52 (3), 221-232.
- Lee, E.J. (2011). Targeted bisulfite sequencing by solution selection and massively parallel sequencing. *Nucleic Acids Research*, 39(19), e127, doi:10.1093/nar/gkr598
- Papageorgiou, EA. (2009). Sites of differential DNA methylation between placenta and peripheral blood. *Am J Pathol*, 174 (5), 1609-1618.
- Ferguson, A.A. et al. (2009). Retrofitting ampicillin resistant vectors by recombination for use in generating *C. elegans* transgenic animals by bombardment. *Plasmid*, 62, 140-145.

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Buffer Preparation

- ✓ ***Before starting:*** Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate. Add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 3,000 - 5,000 x g.

1. Add 2-7 volumes of **DNA Binding Buffer** to each volume of DNA sample (see table below). Mix briefly by vortexing.

| Application | DNA Binding Buffer : Sample | Example |
|--|-----------------------------|-----------------|
| Plasmid, genomic DNA (>2 kb) | 2 : 1 | 200 µl : 100 µl |
| PCR product, DNA fragment | 5 : 1 | 500 µl : 100 µl |
| ssDNA (e.g. cDNA, M13 phage ¹) | 7 : 1 | 700 µl : 100 µl |

For efficient recovery of high molecular weight DNA (> 20kb to > 200kb), use the **Genomic DNA Clean & Concentrator™ (Cat. Nos. D4010, D4011)**.

2. Transfer sample mixtures to the wells of a **Zymo-Spin™ I-96 Plate²** mounted on a **Collection Plate**.
3. Centrifuge for 5 minutes until sample mixtures have been completely filtered. Discard the flow-through.
4. Add 300 µl **DNA Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 minutes. Repeat wash step, but centrifuge for 15 minutes. (Alternatively, one wash can be performed using 600 µl **Wash Buffer**).
5. Add ≥ 10 µl **DNA Elution Buffer³** or water⁴ directly to the column matrix in each well. Transfer the **Zymo-Spin™ I-96 Plate** onto an **Elution Plate** and centrifuge for 3 minutes to elute the DNA.

Ultra-pure DNA is now ready for use.

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

Notes:

¹ Centrifuge phage-infected bacterial culture at 8,000 x g for 1 minute prior to mixing an aliquot of the phage-containing supernatant with the **DNA Binding Buffer**.

² The capacity of each well of the **Zymo-Spin™ I-96 Plate** is approximately 1.1 ml. The capacity of each well of the **Collection Plate** is approximately 800 µl. Therefore, it may be necessary to load and spin the plate multiple times if a sample has a volume larger than 800 µl.

³ **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

⁴ Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Waiting 1 minute prior to elution may improve the yield of larger (> 6 kb) DNA. For even larger DNA (> 10 kb), the total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

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For the clean-up of short cDNAs or ESTs (≥ 16 nt), we recommend the **Oligo Clean & Concentrator™** (Cat. Nos. D4060, D4061).

Appendix A: ssDNA Purification

cDNA clean-up

The DCC™ kit can be used to effectively clean and concentrate cDNA (>500 nt) following reverse transcription (RT) in the presence/absence of fluorescent dyes. Unincorporated free nucleotides and fluorescent derivatives are efficiently removed using the DCC™, and the recovered cDNA may be used directly for microarray analysis, second-strand cDNA synthesis, or indirect labeling with a fluorescent dye such as NHS ester Cy3 or Cy5.

Hydrolysis

1. Add 10 μ l 0.5 M EDTA and 10 μ l 1 N NaOH to 50 μ l of RT reaction.

The volumes of EDTA and NaOH should be scaled proportionally depending on the Starting volume of the RT reaction.

2. Incubate at 65°C for 15 minutes.

Clean-up

1. Add 490 μ l (7 volumes) of **DNA Binding Buffer** to the hydrolysis reaction above. Mix well.

Neutralization (pH) following RNA hydrolysis is not necessary as the **DNA Binding Buffer** will effectively neutralize the NaOH added to the reaction.

2. Continue with *Step 2* of the Protocol on page 4.

M13 phage ssDNA purification

1. Centrifuge phage-infected bacterial culture at 8,000 x g for 1 minute
2. Transfer 100 μ l of phage-containing supernatant to a 1.5 ml microcentrifuge tube and add 700 μ l (7 volumes) of **DNA Binding Buffer**. Mix briefly by vortexing.

Increased supernatant volumes may be processed by proportionally increasing the amount of **DNA Binding Buffer** added to the sample.

3. Continue with *Step 2* of the Protocol on page 4.

Appendix B: Troubleshooting

Low Recovery

- **Improperly Prepared/Stored DNA Wash Buffer**

Make sure ethanol has been added to the **DNA Wash Buffer** concentrate. Cap the bottle tightly to prevent evaporation over time.

- **Addition of DNA Elution Buffer**

Add elution buffer directly to the well matrix and not to the walls of the well. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb.

- **Incomplete Elution**

1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) and incubate for several minutes prior to elution.
2. Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A_{260}/A_{230} Ratios

- **96-Well Tip Contaminated**

When removing the Zymo-Spin I-96 plate from the Collection Plate, be careful that the tips of the 96-well plate does not come into contact with the flow-through. Trace amounts of salt from the flow-through can contaminate a sample resulting in low A_{260}/A_{230} ratios. Ethanol contamination from the flow-through can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up with the DCC™, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**

Most loading dyes do not contain EDTA and will acidify ($\text{pH} \leq 4$) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Ordering Information

| Product Description | Catalog No. | Kit Size (Preps.) |
|---|----------------|-------------------|
| DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) <i>Supplied with uncapped columns</i> | D4003 D4004 | 50 200 |
| DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) <i>Supplied with capped columns</i> | D4013 D4014 | 50 200 |
| ZR-96 DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg DNA per well) | D4023 D4024 | 2 x 96 4 x 96 |
| DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) <i>Supplied with uncapped columns</i> | D4005 D4006 | 50 200 |
| DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) <i>Supplied with capped columns</i> | D4033 D4034 | 50 200 |
| DNA Clean & Concentrator™-100 (for purification of up to 100 µg DNA per prep.) | D4029 D4030 | 25 50 |
| DNA Clean & Concentrator™-500 (for purification of up to 500 µg DNA per prep.) | D4031 D4032 | 10 20 |
| Oligo Clean & Concentrator™ (for purification of up to 5 µg of oligonucleotides per prep.) | D4060 D4061 | 50 200 |
| Genomic DNA Clean & Concentrator™ (for purification of up to 10 µg genomic DNA per prep.) | D4010 D4011 | 25 100 |


Refer to Page 3 for column design specifics in each kit.

| For Individual Sale | Catalog No. | Size |
|--------------------------------------|--------------------------|-----------------|
| DNA Binding Buffer | D4003-1-L D4004-1-L | 50 ml 100 ml |
| DNA Wash Buffer (concentrate) | D4003-2-24 D4003-2-48 | 24 ml 48 ml |
| DNA Elution Buffer | D3004-4-10 D3004-4-16 | 10 ml 16 ml |
| Zymo-Spin™ I-96 Plate | C2004-2 | 2 plates |
| Collection Plate | C2002 | 2 plates |
| Elution Plate | C2003 | 2 plates |

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DNA PURIFICATION

What is Clean-Spin™ Technology?
 The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

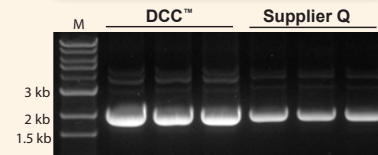
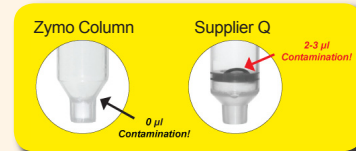


Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small ($\geq 6 \mu\text{l}$) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

| Product | Size (Cat. No.) |
|-----------------------------------|--|
| DNA Clean & Concentrator™-5 | 50 Preps. (D4013) 200 Preps. (D4014) |
| ZR-96 DNA Clean & Concentrator™-5 | 2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024) |
| Genomic DNA Clean & Concentrator™ | 25 Preps. (D4010) 100 Preps. (D4011) |



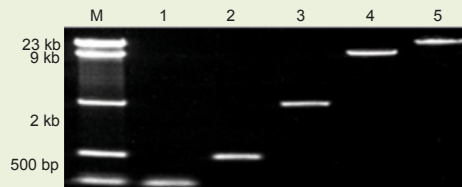
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q.

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in $\geq 6 \mu\text{l}$.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

| Product | Size (Cat. No.) |
|--|---|
| Zymoclean™ Gel DNA Recovery Kit | 50 Preps. (D4001) 200 Preps. (D4002) |
| Zymoclean™ Large Fragment DNA Recovery Kit | 25 Preps. (D4045) 100 Preps. (D4046) |



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli*.
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

Easy, Pellet-free Procedure: Add Lysis Buffer *Directly* to Bacterial Culture

| Product | Size (Cat. No.) |
|-----------------------------|--------------------|
| Zyppy™ Plasmid Miniprep Kit | 50 Preps. (D4036) |
| | 100 Preps. (D4019) |
| | 400 Preps. (D4020) |
| | 800 Preps. (D4037) |



RNA PURIFICATION

Get RNA *directly* from TRIZOL® without phase separation

What is Clean-Spin™ Technology?
 The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

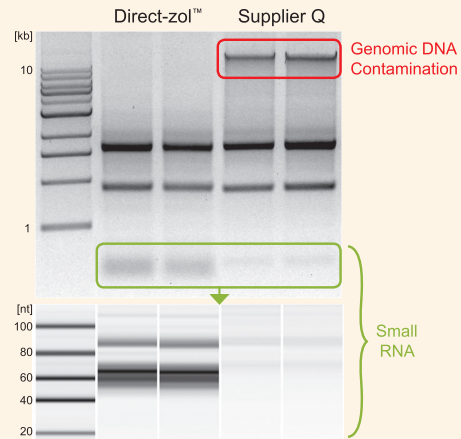
Direct-zol™ RNA

- ✓ For purification of high-quality small and large RNA *directly* from TRIZOL®, TRI Reagent®, or similar.
- ✓ Bypasses phase separation and precipitation procedures allowing for unbiased recovery of miRNA

| Product | Size (Cat. No.) |
|--------------------------|---------------------|
| | 50 Preps. (R2050) |
| Direct-zol™ RNA MiniPrep | 50 Preps. (R2051)* |
| | 200 Preps. (R2052) |
| | 200 Preps. (R2053)* |

96-well and MagBead formats also available!

DNase I included in all kits.
 * Supplied with TRI-Reagent®



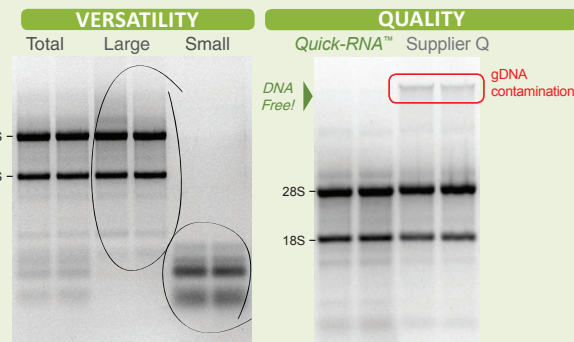
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is **DNA-free**.

Isolate DNA-free RNA from 1 to 10⁷ cells in minutes

Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10⁷ cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

| Product | Size (Cat. No.) |
|----------------------|--|
| Quick-RNA™ MicroPrep | 50 Preps. (R1050) 200 Preps. (R1051) |
| Quick-RNA™ MiniPrep | 50 Preps. (R1054) 200 Preps. (R1055) |
| ZR-96 Quick-RNA™ | 2 x 96 Preps. (R1052) 4 x 96 Preps. (R1053) |



Isolate total, large, or small RNA with the Quick-RNA™ kit.

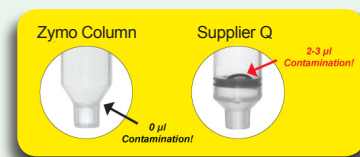
RNA is DNA-free using the Quick-RNA™ kit

Purify RNA from enzymatic and labeling reactions in 5 minutes

RNA Clean & Concentrator™

- ✓ Recover ultra-pure RNA in small (≥6 µl) elution volumes.
- ✓ Compatible with TRIZOL®, phenol, chloroform, and RNase inhibitors (RNAIater®).
- ✓ RNA is ideal for RT-PCR, q-PCR, hybridization, arrays, RNA interference, etc.

| Product | Size (Cat. No.) |
|---------------------------------|---|
| RNA Clean & Concentrator™-5 | 50 Preps. (R1015) 200 Preps. (R1016) |
| RNA Clean & Concentrator™-25 | 50 Preps. (R1017) 100 Preps. (R1018) |
| ZR-96 RNA Clean & Concentrator™ | 2x96 well plates (R1080) |
| DNA-Free RNA Kit™ | 50 Preps. (R1013) 200 Preps. (R1014) |



OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

Competent cells for transformations *without* heat shock!

Mix & Go! Pre-made Competent *E. Coli*

- ✓ High efficiency: 10^8 - 10^9 transformants/ μ g plasmid DNA
- ✓ Just *Mix & Go!* Simply add DNA then spread. Transformation in as little as 20 seconds!

| Product | Size (Cat. No.) |
|--|---|
| Zymo 5 α (Same as DH5 α) | 10 x 100 μ l aliquots (T3007) 96 x 50 μ l aliquots (T3009) 96 x 50 μ l aliquots PCR-plate (T3010) |
| Zymo 10B (Same as DH10B) | 10 x 100 μ l aliquots (T3019) 96 x 50 μ l aliquots (T3020) |
| JM109 | 10 x 100 μ l aliquots (T3003) 96 x 50 μ l aliquots (T3005) |
| HB101 | 10 x 100 μ l aliquots (T3011) 96 x 50 μ l aliquots (T3013) |
| C600 | 10 x 100 μ l aliquots (T3015) |
| TG1 | 10 x 100 μ l aliquots (T3017) |

- ✓ No heat shock
- ✓ No incubations
- ✓ No outgrowth
- ✓ No wait!!!

Mix & Go Competent cells

E. coli + DNA

Mix & Go!
for 20 second*
transformations!

Others

E. coli + DNA

Ice 45 min.

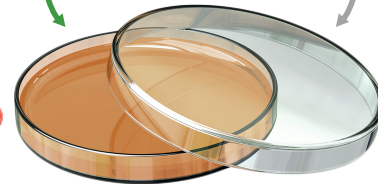
42°C 2 min. (Heatshock)

Place on Ice

Add SOC

Spin to Concentrate Cells

Remove Supernatant

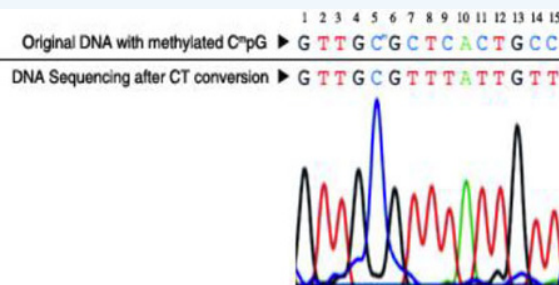


* For Ampicillin selection only.

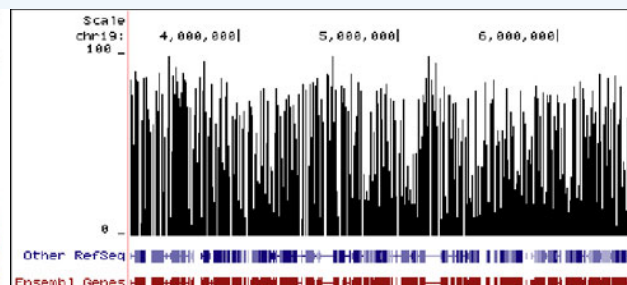
The fastest method for complete bisulfite conversion of DNA

EZ DNA Methylation-Lightning™ Kits

- ✓ The next generation of bisulfite conversion technology by the most cited provider in the industry
- ✓ Guarantees high conversion efficiencies of cytosine (>99.5%)
- ✓ Maintains the highest template integrity following bisulfite conversion
- ✓ Recovered DNA is ideal for PCR, MSP, array, bisulfite, and next-generation sequencing.

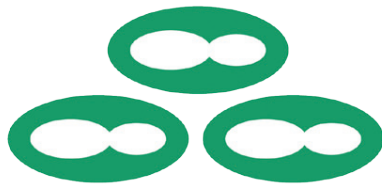


DNA Sequencing Results Following Bisulfite Treatment



Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS)

| Product | Size (Cat. No.) |
|---|---|
| EZ DNA Methylation-Lightning™ Kit | 50 rxns. (D5030) 200 rxns. (D5031) |
| EZ-96 DNA Methylation-Lightning™ Kit | Shallow-Well 2 x 96 rxns. (D5032) Deep-Well 2 x 96 rxns. (D5033) |
| EZ-96 DNA Methylation-Lightning™ MagPrep | 4 x 96 rxns. (D5046) 8 x 96 rxns. (D5047) |



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The Beauty of Science is to Make Things Simple