ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zymoclean™ Large Fragment DNA Recovery Kit

Catalog Nos. **D4045 & D4046**

Highlights

- Quick (15 minute) spin column recovery of large-sized DNA (e.g., genomic, plasmid (BAC/PAC), viral, phage, etc.) from agarose gels. *No messy precipitations!*
- Unique column design for low volume (≥10 µl) elution of ultra-pure, high-yield DNA.
- Eluted DNA is well suited for use in PCR, sequencing, endonuclease digestion, ligation, etc.

Contents

ZYMO RESEARCH CORP.

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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

Zymoclean™ Large Fragment DNA Recovery Kit (Kit Size)	**D4045** (25 Preps.)	**D4046** (100 Preps.)	**Storage Temperature**
ADB	50 ml	100 ml	Room Temp.
DNA Wash Buffer[1]	6 ml	24 ml	Room Temp.
DNA Elution Buffer	1 ml	4 ml	Room Temp
Zymo-Spin™ IC-XL Columns	25	100	Room Temp.
Collection Tubes	50	200	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.

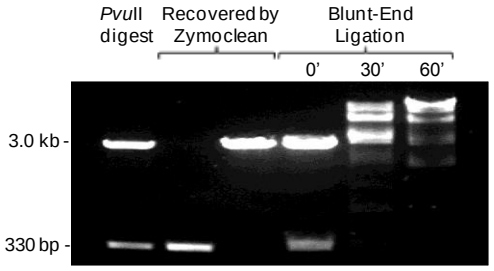
Specifications

- **DNA Purity** – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- **DNA Size Limits** – From ~50 bp to >200 kb.
- **DNA Recovery** – Typically, up to 10 µg total DNA per column can be eluted into ≥10 µl of low salt **DNA Elution Buffer** or water. Recovery of DNA ranges from 70-95%.
- **Sample Sources** – DNA in excised agarose gel slices.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

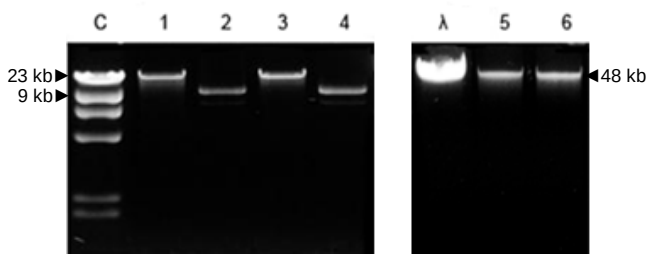
Product Description

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

The **Zymoclean™ Large Fragment DNA Recovery Kit** provides a streamlined method for the rapid purification and concentration of high-quality large-sized DNA from agarose gels. Simply add the specially formulated **Agarose Dissolving Buffer (ADB)** to the gel slice containing a DNA sample, let dissolve, and then transfer to the supplied **Zymo-Spin™ IC-XL Column**. There is no need for organic denaturants or chloroform. Instead, the product utilizes unique spin column technology to yield high-quality, purified DNA in just minutes. DNA purified using the **Zymoclean™ Large Fragment DNA Recovery Kit** is ideal for PCR, sequencing, endonuclease digestion, ligation, etc. The entire procedure typically takes about 15 minutes.



Blunt-ended ligation of DNA fragments purified using the Zymoclean™ Large Fragment DNA Recovery Kit. Fragments from plasmid DNA digested with PvuII were purified, then mixed and ligated for the times indicated in the figure (above).



DNA recovery (lanes 1-6) from HindIII-digested lambda DNA (lane C) and lambda DNA (λ) using the Zymoclean™ Large Fragment DNA Recovery Kit. Lanes **1**, **3**: 23 kb and **2**, **4**: 9 kb bands, respectively. Lanes **5**, **6**: ~48 kb bands.

Zymoclean™ products are offered in single column (uncapped or capped column) or 96-well format. The **Zymoclean™ Gel DNA Recovery Kit** is ideal for purifying DNA from 50 bp – 10 kb with recovery into ≥ 6 µl.

Available Formats

	Uncapped Column	Capped Column	96- well	Capped Column
				
			High-throughput	For Large DNA
Capacity	5 µg/ prep.	5 µg/ prep.	5 µg/ well.	10 µg/ prep.
Elution Vol.	≥ 6 µl	≥ 6 µl	≥ 10 µl	≥ 10 µl
Cat. Nos.	D4001, D4002	D4007, D4008	D4021, D4022	D4045, D4046

Buffer Preparation

- ✓ *Before starting:* Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Protocol[1]

All centrifugation steps should be performed between 11,000 - 16,000 x g.

1. Excise the DNA fragment[1] from the agarose gel using a razor blade or scalpel and transfer it to a 1.5 ml microcentrifuge tube.
2. Add 3 volumes of **ADB** to each volume of agarose excised from the gel (*e.g.* for 100 µl (mg) of agarose gel slice add 300 µl of **ADB)**.
3. Incubate at 37-55 °C for 5-10 minutes until the gel slice is completely dissolved[2].
4. Transfer the melted agarose solution to a **Zymo-Spin™ Column** in a **Collection Tube**.
5. Centrifuge for 1 minute. Discard the flow-through[3].
6. Add 200 µl of **DNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.
7. Add ≥10 µl **DNA Elution Buffer**[4] or water[5] directly to the column matrix and wait for 1 minute. Place column into a 1.5 ml tube and centrifuge for 30 seconds to elute DNA.

   Ultra-pure DNA is now ready for use.

Notes:

[1] The amount of agarose excised from the gel should be as small as possible.

[2] Do not incubate above 60°C. It is important that the gel slice dissolves completely. This can be facilitated by gentle mixing during the incubation.

[3] Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

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

[5] Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. The total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

Troubleshooting

Low Recovery

- **Ensure Agarose is Fully Dissolved**
  There may be small globules of undissolved agarose in the sample, containing DNA inaccessible for recovery. Undissolved agarose can also inhibit DNA recovery by clogging the column and leeching salts into the eluate.
- **Gel Dissolved at Temperatures Above 60 °C**
  If dissolved at a higher temperature, DNA may be denatured affecting recovery. For optimal results, dissolve the gel slice between 37-55 °C.
- **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 column matrix, not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10kb.
- **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) to the column 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 A260/A230 ratio

- **Column tip contaminated**
  When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in a low A_{260}/A_{230} ratio. Ethanol contamination from the flowthrough can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**
  Most loading dyes do not contain EDTA and will acidify (pH ≤ 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.)
Zymoclean™ Gel DNA Recovery Kit *Supplied with **uncapped columns***	D4001 D4002	50 200
Zymoclean™ Gel DNA Recovery Kit *Supplied with **capped columns***	D4007 D4008	50 200
Zymoclean™ Large Fragment Gel DNA Recovery Kit *Supplied with **capped columns***	D4045 D4046	25 100
ZR-96 Zymoclean™ Gel DNA Recovery Kit *Supplied with 96-well plates*	D4021 D4022	2 x 96 4 x 96

Refer to Page 2 for column design specifics in each kit.

For Individual Sale	Catalog No.	Size
ADB	D4001-1-50 D4001-1-100	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24	6 ml 24 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10	1 ml 4ml 10 ml
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1000 tubes

DNA PURIFICATION

What is Clean-Spin™ Technology?

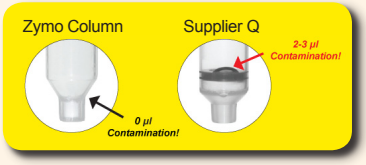
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

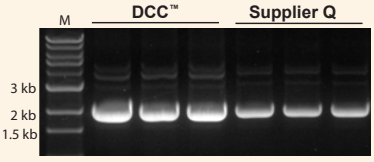
Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) 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)





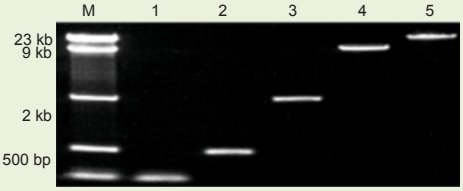
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ 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

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.

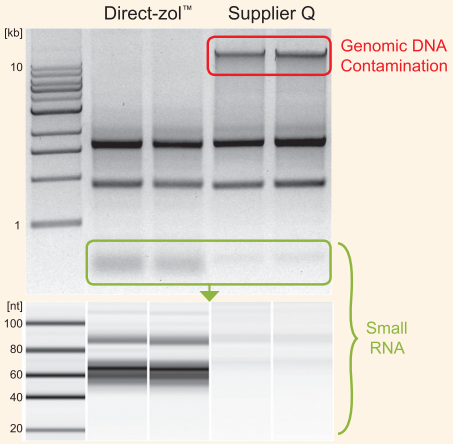
Get RNA *directly* from TRIzol® without phase separation

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.)
Direct-zol™ RNA MiniPrep	50 Preps. (R2050) 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®*



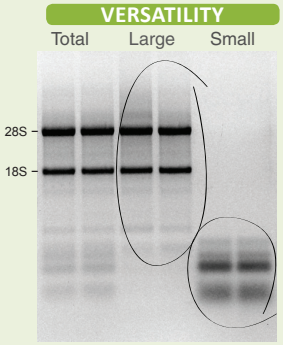
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

Isolate DNA-free RNA from 1 to 10^7 cells in minutes

Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10^7 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.

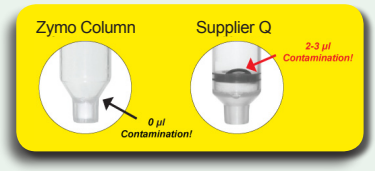
RNA is DNA-free using the *Quick-RNA*™ kit

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, choloform, and RNase inhibitors (RNAlater®).
- ✓ 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)



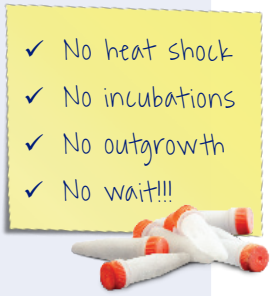
OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

Competent cells for transformations *without* heat shock!

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

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

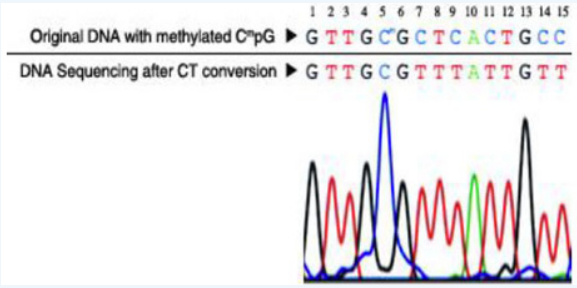
Product	Size (Cat. No.)
Zymo 5α (Same as DH5α)	10 x 100 µl aliquots (T3007) 96 x 50 µl aliquots (T3009) 96 x 50 µl aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 µl aliquots (T3019) 96 x 50 µl aliquots (T3020)
JM109	10 x 100 µl aliquots (T3003) 96 x 50 µl aliquots (T3005)
HB101	10 x 100 µl aliquots (T3011) 96 x 50 µl aliquots (T3013)
C600	10 x 100 µl aliquots (T3015)
TG1	10 x 100 µl aliquots (T3017)



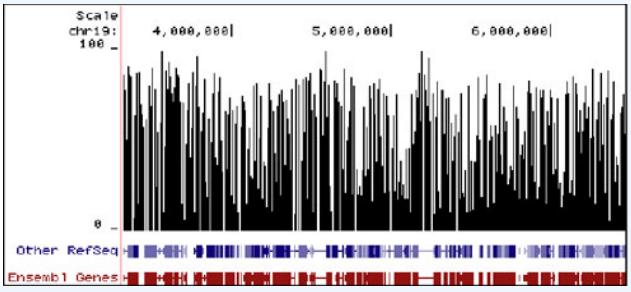
The fastest method for complete bisulfite conversion of DNA

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.

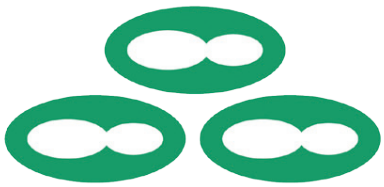


DNA Sequencing Results Following Bisulfite Treatment



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 Deep-Well	2 x 96 rxns. (D5032) 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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