ZYMO RESEARCH

RNA Purification Made Simple™

# ZR-96 Oligo Clean & Concentrator™

Clean-up DNA/RNA oligos from any reaction

## Highlights

- High-throughput (96-well), spin-plate clean-up of ultra-pure DNA and RNA oligonucleotides.
- Complete removal of dyes, salts, enzymes, nucleotides and short oligos.
- Eluted DNA/RNA (≥ 10 µl) is ready for hybridization, sequencing, PCR, ligation, etc.

Catalog Numbers:
D4062, D4063

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

# Table of Contents

# Product Contents

| ZR-96 Oligo Clean & Concentrator™ | D4062 (2 x 96 prep) | D4063 (4 x 96 prep) |
|---|---|---|
| Oligo Binding Buffer | 40 ml | 40 ml (x2) |
| DNA Wash Buffer (concentrate)[1] | 48 ml | 48 ml (x2) |
| Zymo-Spin™ I-96 Plate | 2 | 4 |
| Collection Plate | 4 | 8 |
| Elution Plate | 2 | 4 |
| 96-Well Cover Foil | 2 | 4 |
| Instruction Manual | 1 | 1 |

**Storage Temperature** - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Prior to use, add 192 ml 100% ethanol (208 ml of 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate .

# Specifications

- **Sample Sources –** Enzymatic reactions mixtures containing oligonucleotides ≥ 16 nt (radioactive-, biotin-, DIG-labeled, etc.)
- **Size Limits –** For oligonucleotides ≥ 16 nt, up to 23 kb.
- **Purity –** $A_{260}/A_{280}$ & $A_{260}/A_{230}$ > 1.8. DNA/RNA is ready for hybridization, sequencing, ligation, PCR and etc.
- **Binding Capacity –** 10 µg ssDNA/RNA or 5 µg dsDNA per well, with a typical recovery of > 90% (**Zymo-Spin™ I-96 Plate**).
- **Elution Volume –** ≥ 10 µl (water not provided).
- **Detergent Tolerance –** ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.
- **Equipment Needed** (user provided) **–** Centrifuge with a spin-plate rotor.

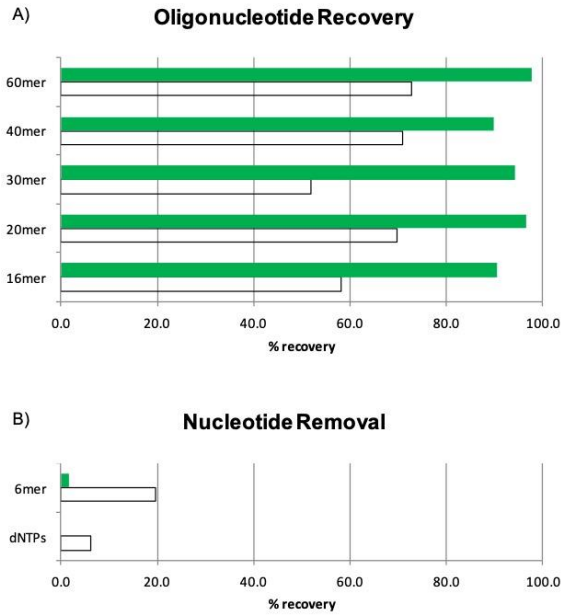
# Applications

- **Isotope and Dye Removal –** Efficiently removes unincorporated fluorescent (i.e., AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, etc.) and radiolabeled dNTP derivatives from DNA following in vitro labeling reactions.
- **DNA Fragment Clean-up from Enzymatic Reactions –** Desalting of DNA with the removal of DNA polymerases, modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, etc.
- **Post-Reverse Transcription (RT) and cDNA Clean-up –** Purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template (page 6).

# Product Description

The **ZR-96 Oligo Clean & Concentrator™** kit provides a streamlined method for high-throughput (96-well), efficient recovery and clean-up of DNA and RNA oligonucleotides ≥16 nt from labeling (radioactive, biotin, DIG, *etc.*) and other enzymatic reactions. Unincorporated nucleotides, short oligos, dyes, enzymes, and salts are effectively removed by the clean-up procedure.

There is no need for organic denaturants or chloroform. Instead, the kit features Zymo-Spin™ plate technology and employs a single-buffer system that allows for efficient oligonucleotide adsorption to the matrix of each well in the **Zymo-Spin™I-96 Plate**. Oligonucleotide is washed and concentrated into a small volume of water (≥ 10 µl). Purified oligonucleotide is suitable for hybridization, gel shift assays, enzymatic reactions, ligation, sequencing, microarray analysis, *etc*.




**Oligo Clean & Concentrator™** facilitates > 90% recovery of ssDNA oligonucleotides (A) and efficient short oligo and nucleotide removal (B).

# Protocol

The protocol consists of: (I) Buffer Preparation and (II) DNA/RNA Clean-Up

## (I) Buffer Preparation

- ✓ Prior to use, add 192 ml 100% ethanol (208 ml of 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

## (II) DNA/RNA Clean-up

- ✓ Perform all steps at room temperature and centrifugation at ≥ 2,500 x g for 5 minutes, unless specified.
- ✓ Scale up the volumes proportionally (steps 1-2), if needed.
- ✓ Do not use the **96-Well Plate Cover Foil** on the spin-plate during DNA/RNA Clean-Up. If necessary, use an Air Permeable Sealing Cover (#C2011-8); sold separately.

1. Add 100 µl **Oligo Binding Buffer** to 50 µl sample[1].

2. Add 400 µl ethanol[2] (95-100%) and mix well by pipetting.

3. Transfer the sample to each well of the **Zymo-Spin™ I-96 Plate**[3] mounted on a **Collection Plate** and centrifuge. Discard the flow-through[4].

4. Add 750 µl **DNA Wash Buffer** to the column and centrifuge. Then centrifuge again to ensure complete removal of the wash buffer. Carefully, transfer the plate onto an **Elution Plate**.

5. Add 25 µl water[5] directly to the matrix of each well and centrifuge.

   Alternatively, for highly concentrated DNA/RNA use ≥ 10 µl elution.

   The eluted oligonucleotide can be used immediately or stored frozen.
   Use the **96-Well Cover Foil** to prevent the eluate from evaporation.

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1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
2 For DNA/RNA ≥ 80 nt, only 200 µl ethanol is required.
3 To process samples >700 µl, **Zymo-Spin™** plate may be reloaded.
4 For **radioactive samples**, transfer the column into a new **Collection Tube** and discard the tube containing the radioactive flow-through appropriately.
5 Alternatively, TE buffer can be used for elution (if required).

# Appendices

### cDNA Clean-Up following Reverse Transcription (RT)

The **Oligo Clean & Concentrator** can be used to effectively clean and concentrate first-strand cDNA following reverse transcription (RT) and hydrolysis. The **Oligo Binding Buffer** will neutralize the hydrolysis reaction and the recovered cDNA may be used directly for microarray analysis. etc.

**Hydrolysis Reaction**: To each 30-50 µl RT reaction, add 10 µl 0.5 M EDTA and 10 µl 1 M NaOH. Then mix and incubate at 65°C for 15 minutes. Proceed to the DNA/RNA Clean-Up protocol, page 5.

# Format Compatibility

To adjust binding capacity, simply replace the provided columns with the formats indicated below and follow their respective elution volumes.

| Format | Zymo-Spin™ I & IC | Zymo-Spin™ II & IICR | Zymo-Spin™ III & IIICG | Zymo-Spin™ I-96 Plate |
|---|---|---|---|---|
| Item Image |  |  |  |  |
| Kit Name | OCC™ | N/A | N/A | ZR-96 OCC™ |
| Capacity | 10 µg ssDNA/RNA or 5 µg dsDNA / prep. | 50 µg ssDNA/RNA or 25 µg dsDNA / prep. | 100 µg ssDNA/RNA or 50 µg dsDNA / prep. | 10 µg ssDNA/RNA or 5 µg dsDNA / prep. |
| Elution Vol. | ≥ 6 µl | ≥ 25 µl | ≥ 50 µl | ≥ 10 µl |
| Column Cat. Nos. | C1003-50, C1004-50 | C1008-50, C1078-50 | C1005-50, C1006-50-G | C2004 |

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| **ZR-96 Oligo Clean & Concentrator™** | D4062<br>D4063 | 2 x 96 preps.<br>4 x 96 preps. |

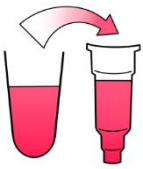
| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **Oligo Binding Buffer** | D4060-1-10<br>D4060-1-40 | 10 ml<br>40 ml |
| **DNA Wash Buffer** (concentrate) | D4003-2-24<br>D4003-2-48 | 24 ml<br>48 ml |
| **Zymo-Spin™ I-96 Plate** | C2004 | 2 plates |
| **Collection Plate** | C2002 | 2 plates |
| **Elution Plate** | C2003 | 2 plates |

# Complete Your Workflow

✓ For tough-to-lyse samples in TRIzol, use ZR BashingBead Lysis Tubes:

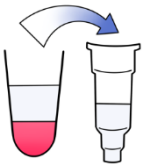
| **ZR BashingBead Lysis Tubes** | |
|---|---|
| 2.0 mm beads #S6003 | For plant/animal tissue |
| 0.1 + 0.5 mm beads #S6012 | For microbes |
| 0.1 + 2.0 mm beads #S6014 | For microbes in tissue/insects |

✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



| **Direct-zol RNA kits** | |
|---|---|
| Microprep #R2060-R2063 | From 1 cell and up |
| Miniprep #R2050-R2053 | Up to 50 ug RNA |
| Miniprep Plus #R2070-R2073 | Up to 100 ug RNA |
| 96-well #R2054-R2057 | Spin-plate |
| MagBeads #R2100-R2105 | Automatable (Tecan, Hamilton, Kingfisher, etc.) |

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):



| **RNA Clean & Concentrator kit** | |
|---|---|
| #R1013-R1014 | DNase I Set included |

✓ For NGS:

| **Zymo-Seq RiboFree Total RNA Library Prep kit** | |
|---|---|
| #R3000 | 12 preps |
| #R3003 | 96 preps |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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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