ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Genomic DNA II Kit™

Catalog Nos. **D3006, D3007, D3024, & D3025**

Highlights

- Easy purification of high quality DNA from whole blood, plasma, serum, body fluids, buffy coat, lymphocytes, tissue, swabs or cultured cells in less than 15 minutes using innovative *Fast-Spin* column technology.
- Compatible with commonly used anticoagulants (i.e., EDTA, heparin, citrate).
- Excludes the use of Proteinase K and organic denaturants.
- Isolated DNA is ideal for PCR, endonuclease digestion, bisulfite conversion/methylation detection, sequencing, genotyping, etc.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are not satisfied with this product please call 1-888-882-9682.

Product Contents

ZR Genomic DNA II Kit™ (Kit Size)	**D3006** (uncapped) **D3024** (capped) (50 Preps.)	**D3007** (uncapped) **D3025** (capped) (200 Preps.)	**Storage Temperature**
Genomic Lysis Buffer	50 ml	2 x 100 ml	Room Temp.
DNA Pre-Wash Buffer	15 ml	50 ml	Room Temp.
g-DNA Wash Buffer	50 ml	100 ml	Room Temp.
DNA Elution Buffer	4 ml	10 ml	Room Temp.
Zymo-Spin™ Columns*	50 columns	200 columns	Room Temp.
Collection Tubes	100 tubes	400 tubes	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.

* Kits **D3006** and **D3007** are supplied with uncapped columns (**Zymo-Spin™ IIN**), whereas kits **D3024** and **D3025** are supplied with capped columns (**Zymo-Spin™ IIC**).

Specifications

- **Sample Sources** – Whole blood, plasma, or serum from humans, mice, rats, etc. Also, tissue, cells from culture, buccal cells, as well as a variety of biological liquids are effectively processed using this kit.
- **DNA Purity** – High-quality DNA is eluted with **DNA Elution Buffer** or water. DNA is especially well suited for PCR and other downstream applications. Typical absorption indices are $A_{260}/A_{280} > 1.8$.
- **DNA Size Limits** – Capable of recovering genomic DNA up to and above 40 kb. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.
- **DNA Recovery** – Typically, up to 25 µg total DNA is eluted into ≥ 50 µl (30 µl minimum) **DNA Elution Buffer** or water**.** Human whole blood will yield 3 - 7 µg DNA per 100 µl blood sampled. Mammalian tissues yield: 1 - 3 µg DNA per mg skeletal, heart, and brain tissues and 3 - 5 µg DNA per mg liver, kidney and lung tissues.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.
- **Equipment** – microcentrifuge, vortex

Product Description

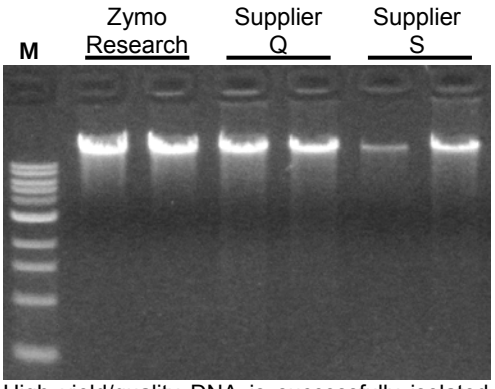
The **ZR Genomic DNA II Kit™** is a simple procedure for the rapid isolation of total DNA (e.g., genomic, mitochondrial, viral) from a variety of biological sample sources. This product has been optimized for maximal recovery of ultra-pure DNA without RNA contamination and is compatible with whole blood (fresh or stored), serum, plasma, buffy coat, solid tissue, bone marrow and buccal cells, cells from culture, and many biological liquid samples. For processing, simply add the specially formulated **Genomic Lysis Buffer** to a sample, vortex, and transfer the mixture to the supplied **Zymo-Spin™ Column**. There is no need for organic denaturants or Proteinase K digestion because of the unique chemistries featured in the kit. Instead, the product features *Fast-Spin* column technology to yield high-quality, purified DNA in just minutes (see below). PCR inhibitors are effectively removed during the purification process. DNA purified using the **ZR Genomic DNA II Kit™** is suitable for PCR, nucleotide blotting, DNA sequencing, endonuclease digestion, bisulfite conversion/methylation analysis, and other downstream applications.

The **ZR Genomic DNA I Kit™** (**D3004, D3005**) and **ZR Serum DNA Kit™** (**D3013**) are recommended for yields >25 µg per prep. Both feature silica beads instead of a spin column.

Zymo Research offers the **EZ DNA Methylation™** (**D5001, D5002, D5003**), **EZ DNA Methylation-Gold™** (**D5005, D5006, D5007, D5008**) **and EZ DNA Methylation-Direct™ (D5020, D5021, D5022, D5023) Kits** for rapid, precise DNA methylation detection.

Ultra-pure DNA is ideal for…

- PCR
- Endonuclease Digestion
- Genotyping
- Bisulfite Conversion & Methylation Analysis



High yield/quality DNA is successfully isolated from porcine whole blood using the **ZR Genomic DNA II Kit™**. Equivalent amounts (100 µl) of blood were processed without Proteinase K using the **ZR Genomic DNA II Kit™** in half the time as compared to the kits from suppliers Q and S. Equal volumes of eluted DNA were then analyzed (in duplicate) in a 0.8% (w/v) TAE/agarose/ethidium bromide gel. The size marker "M" is a 1 kb ladder (Zymo Research).

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

PROTOCOLS

WHOLE BLOOD, SERUM, AND PLASMA SAMPLES

The following is for the purification of DNA from 50 µl whole blood, serum or plasma (the volumes can be adjusted depending on your requirements). Fresh, frozen, or preserved blood (in EDTA, citrate, or heparin) can be used. If material cannot be processed immediately, the sample can be "stabilized" for later processing (as noted below) although the immediate processing of blood samples is recommended.

1. Add 200 µl of **Genomic Lysis Buffer** to 50 µl of blood, serum, or plasma. Mix completely by vortexing 4 - 6 seconds, then let stand 5 minutes at room temperature.

   Note: Add 200 µl Genomic Lysis Buffer to all samples < 50 µl. For samples larger than 50 µl, add a proportional amount of Genomic Lysis Buffer (e.g., for 100 µl blood, add 400 µl Genomic Lysis Buffer).

The column capacity is ~1 ml.

2. Transfer the mixture to a **Zymo-Spin™ Column** in a **Collection Tube**. Centrifuge at 10,000 *x g* for one minute. Discard the **Collection Tube** with the flow through.

3. Transfer the **Zymo-Spin™ Column** to a new **Collection Tube**. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

4. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is > 6.0. Also, the total yield may be improved by eluting the DNA with Elution Buffer or water pre-equilibrated to 60-70°C.

5. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** or water to the spin column. Incubate 2 - 5 minutes at room temperature, then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

Delayed Processing of Blood Samples: The immediate processing of blood with this kit is recommended. However, if blood cannot be processed immediately, samples can be "stabilized" in **Genomic Lysis Buffer** for processing at a later time. To do this, add four volumes of **Genomic Lysis Buffer** to each volume of whole blood (4:1), then vortex. Blood samples mixed with **Genomic Lysis Buffer** can be stored at room temperature for 7 days, 0 - 4ºC for up to 15 days, -20ºC for 2 months, or < -70ºC for many years. Samples stored at ≤ 4ºC should reach room temperature prior to processing. Begin at Step 2 in the standard protocol (above) when purifying DNA from blood samples stabilized in **Genomic Lysis Buffer**.

BUCCAL CELL SAMPLES

Buccal cells can be isolated using a rinse- or swab-based isolation method.

A. **Rinse Method**: Vigorously rinse 10 - 20 ml of saline solution or mouthwash orally for 30 seconds. The more vigorous the rinsing action, the more cells that will be recovered. Spit the saline into a 50 ml tube and pellet the cells at 1,500 rpm for 5 minutes. Discard the supernatant without disturbing the cell pellet. Add 500 µl of **Genomic Lysis Buffer** to the pellet then vortex 4 - 6 seconds, then let stand at room temperature for 5 minutes.

B. **Swab Isolation Method**: Thoroughly rinse mouth out before isolating cells. Brush the inside of the cheek with a buccal swab for 15 seconds (approximately 20 brushes), making sure to cover the entire area of the inner cheek. Rinse the brush into a microcentrifuge tube using 500 µl of **Genomic Lysis Buffer** then vortex 4 - 6 seconds, then let stand at room temperature for 5 minutes.

1. Transfer the mixture to a **Zymo-Spin™ Column** in a **Collection Tube**. Centrifuge at 10,000 *x g* for one minute. Discard the **Collection Tube** with the flow through.

2. Transfer the **Zymo-Spin™ Column** to a new **Collection Tube**. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

3. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

4. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** or water to the spin column. Incubate 2 - 5 minutes at room temperature, then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

SOLID TISSUE SAMPLES

Note: For Proteinase K digested materials (e.g., tailsnips) follow the protocol for **CELL SUSPENSIONS AND PROTEINASE K DIGESTED SAMPLES** (pg. 6). Otherwise…mechanically homogenize up to 20 mg of fresh or frozen tissue in 500 µl of **Genomic Lysis Buffer**. Increase the reagents proportionally if more than 20 mg of solid tissue is used or reduce the reagents to half the volume described in the protocol should the tissue sampled be less than 5 mg.

Soft tissue samples are readily homogenized using our **Squisher™-Single**, **Squisher™-8**, and **Squisher™-96** products.

Typical yields are: 1 - 3 µg DNA per mg skeletal, heart, and brain tissues and 3 - 5 µg per mg liver, kidney, and lung tissues.

1. Centrifuge the lysate at top speed (10,000 *x g*) for 5 minutes. Making sure not to disturb the pelleted debris, transfer the supernatant to a **Zymo-Spin™ Column** in a **Collection Tube** and centrifuge at 10,000 *x g* for one minute. Discard the **Collection Tube** with the flow through.

2. Transfer the **Zymo-Spin™ Column** to a new **Collection Tube**. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

3. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

4. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** or water to the spin column. Incubate 2 - 5 minutes at room temperature, then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20 ºC for future use.

CELL MONOLAYER SAMPLES

Generally, no more than 5 x10^6 cells should be sampled, for larger samples will exceed the binding capacity of the spin column.

The following procedure is designed for up to 1.0 x 10^6 monolayer cells (roughly equal to one well of a 6-well plate or ½ of a T25 flask). Although cell types and culture conditions may vary, the protocol will work with high-density growth cells (e.g., HeLa cells) as well as with low-density growth cells (e.g., neuronal cells). The procedure may be scaled up or down for increases or decreases in the amounts of monolayer cells sampled (see the **Guidelines for Monolayer Cell DNA Isolation** below).

It may be necessary to centrifuge the sample mixture before transferring the supernatant to the **Zymo-Spin™ Column** to remove particulate matter that may clog the column.

1. Trypsinize or manually scrape adherent cells from the growth surface of a culture flask or plate. Centrifuge the cell suspension at approximately 500 x *g* for 5 minutes. Remove the supernatant and add 500 µl of **Genomic Lysis Buffer** directly to the pellet. Resuspend pellet by vortexing 4 - 6 seconds and let stand for 5 minutes at room temperature.

The column capacity is ~1 ml.

2. Transfer the mixture to a **Zymo-Spin™ Column** in a **Collection Tube**. Centrifuge at 10,000 *x g* for one minute. Discard the **Collection Tube** with the flow through.

3. Transfer the **Zymo-Spin™ Column** to a new **Collection Tube**. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

4. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

5. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** or water to the spin column. Incubate 2 - 5 minutes at room temperature, then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20 C for future use.

Guidelines for Monolayer Cell DNA Isolation: The above procedure is designed for the processing of 0.1 - 1.0 x 10^6 cells. However, cell numbers (growth densities) can vary between different cell types. Table 1 (pg. 6) provides an approximation of what can be recovered from different culture containers for high-density growth cells like CV1 and HeLa cells. The table provides a guideline when adjusting reagent volumes used in the protocol. If the cell number is less than 1.0 x 10^5, use half the volume of **Genomic Lysis Buffer** given in the standard protocol (above). If the cell number is greater than 1.0 x 10^6, use double the volume of **Genomic Lysis Buffer**.

Table 1: Culture Plate/Flask Growth Area (cm^2) and Cell Number

Culture Container	Well /Flask Surface Area	Cell Number
96-well plate	0.32 - 0.6 cm^2	4 - 5 x 10^4
24-well plate	2 cm^2	1 - 3 x 10^5
12-well plate	4 cm^2	4 - 5 x 10^5
6 -well plate	9.5 cm^2	0.5 - 1 x 10^6
T25 Culture Flask	25 cm^2	2 - 3 x 10^6
T75 Culture Flask	75 cm^2	0.6 - 1 x 10^7
T175 Culture Flask	175 cm^2	2 - 3 x 10^7

CELL SUSPENSIONS AND PROTEINASE K DIGESTED SAMPLES

The following protocol is designed for up to 200 µl of biological liquid sample including CSF, buffy coat, body fluids (semen), and cell suspensions containing less than 5.0 x 10^6 cells as well as lysates derived from Proteinase K digested samples.

Cells should be processed directly from biological fluids or from suspension in PBS, TE, or compatible buffers.

1. Add 4 volumes of **Genomic Lysis Buffer** to each volume of liquid sample. (For example, for 200 µl of sample, add 800 µl of **Genomic Lysis Buffer**). Mix briefly by vortexing, then let stand at room temperature for 5 minutes.

   Note: For Proteinase K digested material, add 4 volumes of **Genomic Lysis Buffer** to each volume of lysate then mix briefly by vortexing. Centrifuge the mixture at top speed (>10,000 *x g*) for 5 minutes. Transfer up to 1.0 ml supernatant to the Zymo-Spin™ Column in Step 2.

2. Transfer the mixture to a **Zymo-Spin™ Column** in a **Collection Tube**. Centrifuge at 10,000 *x g* for one minute. Discard the **Collection Tube** with the flow through.

The column capacity is ~1 ml.

3. Transfer the **Zymo-Spin™ Column** to a new **Collection Tube**. Add 200 µl of **DNA Pre-Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

4. Add 500 µl of **g-DNA Wash Buffer** to the spin column. Centrifuge at 10,000 *x g* for one minute.

5. Transfer the spin column to a clean microcentrifuge tube. Add ≥ 50 µl **DNA Elution Buffer** or water to the spin column. Incubate 2 - 5 minutes at room temperature, then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

Typical yields from Proteinase K digested tissues are: 1-3 µg DNA per mg skeletal, heart, and brain tissues and 3-5 µg per mg liver, kidney, and lung tissues.

Troubleshooting:

1. DNA degradation: Check for DNase contamination. All reagents and components supplied with the **ZR Genomic DNA II Kit™** are DNase-free. However, DNase contamination could result during the processing of some samples. Check pipets, pipet tips, microcentrifuge tubes, etc., and exercise the appropriate precautions during the DNA purification procedure.

2. DNA is not performing well in subsequent experiments: Ensure the correct volume of **Genomic Lysis Buffer** has been added to the sample. Also, make sure all centrifugation steps are completed for the indicated times and speeds (rcfs). Failure to do so may result in incomplete washing, which may cause salts to be eluted with the DNA affecting quantitation and subsequent experiments including enzymatic processes like PCR.

3. RNA contamination: The buffers and spin columns provided in this kit are designed to efficiently remove RNA during the DNA purification procedure. However, additional RNA removal (e.g., digestion with RNase A) may be necessary for subsequent applications sensitive to trace amounts of RNA.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR Genomic DNA II Kit™ supplied w/ **uncapped** columns	D3006	50 preps.
ZR Genomic DNA II Kit™ supplied w/ **uncapped** columns	D3007	200 preps.
ZR Genomic DNA II Kit™ supplied w/ **capped** columns	D3024	50 preps.
ZR Genomic DNA II Kit™ supplied w/ **capped** columns	D3025	200 preps.

For Individual Sale	Catalog No.	Amount
Genomic Lysis Buffer	D3004-1-50 D3004-1-100	50 ml 100 ml
DNA Pre-Wash Buffer	D3004-5-15 D3004-5-30 D3004-5-50	15 ml 30 ml 50 ml
g-DNA Wash Buffer	D3004-2-50 D3004-2-100	50 ml 100 ml
DNA Elution Buffer	D3004-4-4 D3004-4-10	4 ml 10 ml
Zymo-Spin™ IIN Columns (uncapped)	C1019-50 C1019-250	50 columns 250 columns
Zymo-Spin™ IIC Columns (capped)	C1011-50 C1011-250	50 columns 250 columns
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1,000 tubes

Popular DNA Purification & Analysis Products from Zymo Research

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.	50 200 50 200	**D4003** (uncapped) **D4004** (uncapped) **D4013** (capped) **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.	50 200 50 200	**D4005** (uncapped) **D4006** (uncapped) **D4033** (capped) **D4034** (capped)
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 50	**D4029** **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 20	**D4031** **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 4x96	**D4023** **D4024**
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes	50 200	**D4001** **D4002**
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2x96 4x96	**D4021** **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).	50 100 400	**D4036** **D4019** **D4020**
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in minutes: (alkaline lysis/spin column format and 150 µl minimum elution volume).	25 50	**D4025** **D4026**
Zyppy™ Plasmid Maxiprep Kit	High-purity plasmid DNA purification in minutes: (alkaline lysis/spin column format and 2 ml minimum elution volume).	10 20	**D4027** **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).	100 400	**D3004** **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.	50 200 50 200	**D3006** (uncapped) **D3007** (uncapped) **D3024** (capped) **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 4x96	**D3010** **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.	50 200	**D3015** **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 4x96	**D3017** **D3018**
EZ DNA Methylation™ Kit	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via the chemical-denaturation of DNA using our specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using *Fast-Spin* column technology. Ultra-pure recovered DNA can be used for PCR and bisulfite sequencing applications.	50 200 2x96 2x96	**D5001** **D5002** **D5003** (Shallow-well) **D5004** (Deep-well)
EZ DNA Methylation-Gold™ Kit	Streamlined kit for the conversion of unmethylated cytosines in DNA to uracil via heat-denaturation of DNA using our specially designed CT Conversion Reagent™. DNA is then desulphonated and subsequently cleaned using *Fast-Spin* column technology. Ultra-pure recovered DNA can be used for PCR and bisulfite sequencing applications. *3 hour processing time!*	50 200 2x96 2x96	**D5005** **D5006** **D5007** (Shallow-well) **D5008** (Deep-well)

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