



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 *Quick-gDNA*™

Catalog Nos. D3010, D3011, & D3012

Highlights

- Quick, high throughput (96-well) purification of DNA from whole blood, plasma, serum, body fluids, buffy coat, lymphocytes, tissue, swabs or cultured cells in less than 25 minutes.
- Compatible with commonly used anticoagulants (i.e., EDTA, heparin, citrate).
- Unique extraction technology excludes the use of Proteinase K and organic denaturants.
- Isolated DNA is ideal for PCR, endonuclease digestion, bisulfite conversion/methylation detection, sequencing, genotyping, etc.

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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-96 Quick-gDNA™ (Kit Size)	D3010 (2 x 96)	D3011 (4 x 96)	D3012 (10 x 96)	Storage Temperature
Genomic Lysis Buffer*	100 ml	2 x 100 ml	5 x 100 ml	Room Temp.
DNA Pre-Wash Buffer**	50 ml	2 x 50 ml	5 x 50 ml	Room Temp.
g-DNA Wash Buffer	100 ml	2 x 100 ml	5 x 100 ml	Room Temp.
DNA Elution Buffer	10 ml	2 x 10 ml	50 ml	Room Temp.
Silicon-A™ Plate	2	4	10	Room Temp.
Collection Plate	2	4	10	Room Temp.
Elution Plate	2	4	10	Room Temp.
Instruction Manual	1	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.

* Recommended: Add beta-mercaptoethanol to 0.5%(v/v) i.e., 500 µl per 100 ml.

**A precipitate may have formed in the DNA Pre-Wash Buffer during shipping. To completely resuspend the buffer, incubate the bottle at 30-37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

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. $A_{260}/A_{280} > 1.8$
- **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** – Up to 5 µg/well total DNA is eluted into ≥30 µl **DNA Elution Buffer** or water. Human whole blood will typically 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, centrifuge w/ microplate carriers

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.

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Product Description

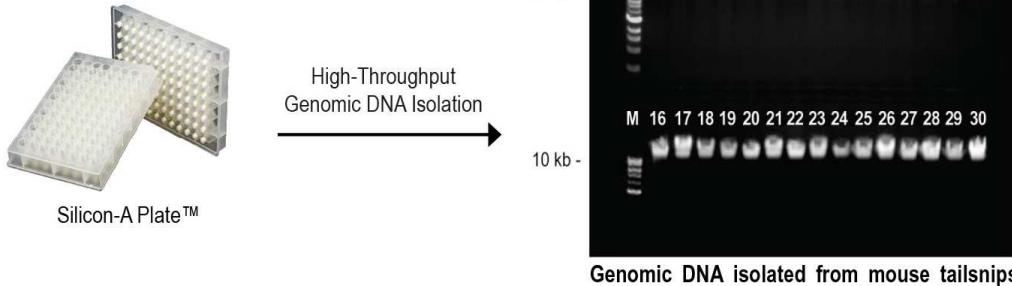
The **ZR-96 Quick-gDNA™** features a simple, high throughput (96-well) procedure for the rapid isolation of total DNA (e.g., genomic, mitochondrial, viral) from a variety of biological sample sources. This product has been optimized for maximal recovery of ultra-pure DNA without RNA contamination and 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 the samples, vortex, and transfer the mixtures to the wells of the supplied **Silicon-A™ Plate**. There is no need for organic denaturants or Proteinase K digestion because of the unique chemistries featured in the kit. Instead, the product yields high-quality, purified DNA in just minutes (see below). PCR inhibitors are effectively removed during the purification process. DNA purified using the **ZR-96 Quick-gDNA™** is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bisulfite conversion/methylation analysis, and other downstream applications.

The **Quick-gDNA™ MicroPrep** (D3020, D3021) and **Quick-gDNA™ MiniPrep** (D3006, D3007, D3024, D3025) provides spin column alternatives for isolation of up to 5 µg and 25 µg DNA/column, respectively.

Zymo Research offers the following for rapid, precise DNA methylation detection...

- 1.) **EZ DNA Methylation™ Kit** (D5001, D5002, D5003, D5004)
- 2.) **EZ DNA Methylation-Gold™ Kit** (D5005, D5006, D5007, D5008)
- 3.) **EZ DNA Methylation-Direct™ Kit** (D5020, D5021, D5022, D5023)



Genomic DNA isolated from mouse tailsnips using the ZR-96 Quick-gDNA™. A total of 30 mouse tailsnips were homogenized with Zymo Research's Squisher-8™ then processed using the ZR-96 Quick-gDNA™. About one third of the number of eluted DNAs was then separated in a 0.8% w/v agarose gel (shown in lanes 1 to 30).

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

Step 1 can be performed directly in the Silicon-A™ Plate. Mixing is performed by pipetting the samples up and down repeatedly.

The capacity of each well of the Silicon-A™ Plate is ~600 µl.

For the inclusion of small DNAs from serum, add 0.3 volumes isopropanol to the mixture. (For example, to a 500 µl mixture of serum and Genomic Lysis Buffer add 150 µl isopropanol.)

Elution of DNA from the Silicon-A™ Plate 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.

Buffer Preparation

- ✓ **Recommended:** Add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5%(v/v) i.e., 250 µl per 50 ml or 500 µl per 100 ml.

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 up to 100 µl (max.) 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 (4:1). Mix completely by vortexing 4-6 seconds, then let stand 5-10 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 (4:1) of Genomic Lysis Buffer (e.g., Add 400 µl Genomic Lysis Buffer to 100 µl blood).
2. Transfer the mixtures to the wells of a **Silicon-A™ Plate** on a **Collection Plate**. Centrifuge at ≥ 2,500 x g (5,000 x g max.) for 5 minutes.
3. Add 200 µl **DNA Pre-Wash Buffer** to each well and centrifuge at ≥ 2,500 x g for 5 minutes. Discard the flow through.
4. Add 300 µl of **g-DNA Wash Buffer** to each well and centrifuge at ≥ 2,500 x g for 5 minutes.
5. Transfer the **Silicon-A™ Plate** onto an **Elution Plate**. Add ≥30 µl **DNA Elution Buffer** or water to each well. Incubate 2-5 minutes at room temperature, then centrifuge at ≥ 2,500 x g for 5 minutes to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20°C for future use.

Delayed Processing (Stabilization) 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 1-2 weeks, 0-4°C for 1-2 months, -20°C for 6 months to a year, or <-70°C for many years. Samples stored at ≤4°C 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 Cells and Swabs

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 μ l of **Genomic Lysis Buffer** to the pellet then vortex 4-6 seconds, then let stand at room temperature for 5-10 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 with 500 μ l of **Genomic Lysis Buffer** into a microcentrifuge tube, vortex 4-6 seconds, and then let stand at room temperature for 5-10 minutes.
1. Transfer the mixtures to the wells of a **Silicon-A™ Plate** on a **Collection Plate**. Centrifuge at $\geq 2,500 \times g$ (5,000 $\times g$ max.) for 5 minutes.
 2. Add 200 μ l **DNA Pre-Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes. Discard the flow through.
 3. Add 300 μ l of **g-DNA Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes.
 4. Transfer the **Silicon-A™ Plate** onto an **Elution Plate**. Add $\geq 30 \mu$ l **DNA Elution Buffer** or water to each well. Incubate 2-5 minutes at room temperature, then centrifuge at $\geq 2,500 \times g$ for 5 minutes to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored $\leq -20^{\circ}\text{C}$ for future use.

The capacity of each well of the Silicon-A™ Plate is $\sim 600 \mu\text{l}$.

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 5 mg of fresh or frozen tissue in 500 μ l of **Genomic Lysis Buffer**.

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

1. Centrifuge the lysates to pellet insoluble material. Making sure not to disturb the pelleted debris, transfer the supernatants to the wells of a **Silicon-A™ Plate** on a **Collection Plate**. Centrifuge at $\geq 2,500 \times g$ (5,000 $\times g$ max.) for 5 minutes.
2. Add 200 μ l **DNA Pre-Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes. Discard the flow through.

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.

3. Add 300 μ l of **g-DNA Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes.
4. Transfer the **Silicon-A™ Plate** onto an **Elution Plate**. Add $\geq 30 \mu$ l **DNA Elution Buffer** or water to each well. Incubate 2-5 minutes at room temperature, then centrifuge at $\geq 2,500 \times g$ for 5 minutes to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored $\leq -20^{\circ}\text{C}$ for future use.

Cell Monolayer Samples

Generally, no more than 1×10^6 cells should be sampled, for larger samples will exceed the binding capacity of the Silicon-A™ Plate

It may be necessary to centrifuge the sample mixtures before transferring the supernatants to the Silicon-A™ Plate to remove particulate matter that may clog the matrices.

The capacity of each well of the Silicon-A™ Plate is $\sim 600 \mu\text{l}$.

*The following procedure is designed for up to 1.0×10^6 (max.) monolayer cells (roughly equal to one well of a 6-well plate). 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).*

1. Trypsinize or manually scrape adherent cells from the growth surface of a culture flask or plate. Centrifuge the cell suspension at approximately $500 \times g$ 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 5-10 minutes at room temperature.

Alternatively: Cells can be lysed directly in the culture container (plate) by removing the medium and adding the Genomic Lysis Buffer directly to the monolayer surface.
2. Transfer the mixtures to the wells of a **Silicon-A™ Plate** on a **Collection Plate**. Centrifuge at $\geq 2,500 \times g$ (5,000 $\times g$ max.) for 5 minutes.
3. Add 200 μl **DNA Pre-Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes. Discard the flow through.
4. Add 300 μl of **g-DNA Wash Buffer** to each well and centrifuge at $\geq 2,500 \times g$ for 5 minutes.
5. Transfer the **Silicon-A™ Plate** onto an **Elution Plate**. Add $\geq 30 \mu\text{l}$ **DNA Elution Buffer** or water to each well. Incubate 2-5 minutes at room temperature, then centrifuge at $\geq 2,500 \times g$ for 5 minutes to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored $\leq -20^{\circ}\text{C}$ for future use.

Guidelines for Monolayer Cell DNA Isolation: The above procedure can be used for the processing of 0.1 - 1.0×10^6 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.

Table 1: Culture Plate/Flask Growth Area (cm²) and Cell Number

Culture Container	Well /Flask Surface Area	Cell Number
96-well plate (each well)	0.32-0.6 cm ²	4-5x10 ⁴
24-well plate (each well)	2 cm ²	1-3x10 ⁵
12-well plate (each well)	4 cm ²	4-5x10 ⁵
6-well plate (each well)	9.5 cm ²	0.5-1x10 ⁶
T25 Culture Flask	25 cm ²	2-3x10 ⁶
T75 Culture Flask	75 cm ²	0.6-1x10 ⁷
T175 Culture Flask	175 cm ²	2-3x10 ⁷

Cell Suspensions and Proteinase K Digested Samples

The following protocol is designed for up to 100 µl of biological liquid sample including CSF, buffy coat, body fluids (semen), and cell suspensions containing less than 1.0x10⁶ cells as well as lysates derived from Proteinase K digested samples.

1. Add 4 volumes of **Genomic Lysis Buffer** to each volume of liquid sample. (For example, for 100 µl of sample, add 400 µl of **Genomic Lysis Buffer**). Mix briefly by vortexing, then let stand at room temperature for 5-10 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 600 µl supernatant to the wells of a Silicon-A™ Plate as in Step 2.
2. Transfer the mixtures to the wells of a **Silicon-A™ Plate** on a **Collection Plate**. Centrifuge at ≥ 2,500 x g (5,000 x g max.) for 5 minutes.

The capacity of each well of the Silicon-A™ Plate is ~600 µl.
3. Add 200 µl **DNA Pre-Wash Buffer** to each well and centrifuge at ≥ 2,500 x g for 5 minutes. Discard the flow through.
4. Add 300 µl of **g-DNA Wash Buffer** to each well and centrifuge at ≥ 2,500 x g for 5 minutes.
5. Transfer the **Silicon-A™ Plate** onto an **Elution Plate**. Add ≥30 µl **DNA Elution Buffer** or water to each well. Incubate 2-5 minutes at room temperature, then centrifuge at ≥ 2,500 x g for 5 minutes to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20°C for future use.

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

The capacity of each well of the Silicon-A™ Plate is ~600 µl.

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 supplied with the **ZR-96 Quick-gDNA™** 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 in this kit are designed to efficiently hydrolyze and remove RNA during the DNA purification procedure.

Ordering Information

Product Description	Cat. No.	Kit Size
Quick-gDNA™ MicroPrep	D3020	50 preps.
	D3021	200 preps.
Quick-gDNA™ MiniPrep w/ uncapped columns	D3006	50 preps.
	D3007	200 preps.
Quick-gDNA™ MiniPrep w/ capped columns	D3024	50 preps.
	D3025	200 preps.
Quick-gDNA™ MidiPrep	D3100	25 preps.
ZR-96 Quick-gDNA™	D3010	2x96 well
	D3011	4x96 well
	D3012	10x96 well

For Individual Sale	Catalog No.	Amount
Genomic Lysis Buffer	D3004-1-50	50 ml
	D3004-1-100	100 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
	D3004-5-30	30 ml
	D3004-5-50	50 ml
g-DNA Wash Buffer	D3004-2-50	50 ml
	D3004-2-100	100 ml
DNA Elution Buffer	D3004-4-4	4 ml
	D3004-4-10	10 ml
	D3004-4-50	50 ml
Silicon-A™ Plate	C2001	2 plates
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates

Popular Products From Zymo Research

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Product	Description	Kit Size (Preps)	Catalog No. (Format)
Fragment DNA Purification			
DNA Clean & Concentrator™-5	Clean and concentrate up to 5 µg DNA into ≥6 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4003 (uncapped) D4004 (uncapped) D4013 (capped) D4014 (capped)
DNA Clean & Concentrator™-25	Clean & concentrate 25 µg of DNA into ≥25 µl elution volume in as little as 2 minutes with no wash residue carryover.	50 200 50 200	D4005 (uncapped) D4006 (uncapped) D4033 (capped) D4034 (capped)
ZR-96 DNA Clean & Concentrator™-5	Quick (15 minute), high-output recovery of up to 5 µg pure DNA into 10-15 µl minimum elution volume allows for highly concentrated DNA.	2x96 4x96	D4023 D4024
Genomic DNA Clean & Concentrator™	Quick (5 minute) clean-up of up to 10 µg high molecular weight DNA (≤200 kb) from any enzymatic reaction or impure preparation without precipitations.	25 100	D4010 (capped) D4011 (capped)
Zymoclean™ Gel DNA Recovery Kit	Purify DNA from high and low-melting agarose gels in minutes	50 200 50 200	D4001 (uncapped) D4002 (uncapped) D4007 (capped) D4008 (capped)
ZR-96 Zymoclean™ Gel DNA Recovery Kit	High-throughput DNA purification from high and low-melting agarose gels.	2x96 4x96	D4021 D4022
Zymoclean™ Large Fragment DNA Recovery Kit	Purify high molecular weight DNA (≤200 kb) from high and low-melting agarose gels in minutes	25 100	D4045 (capped) D4046 (capped)
OneStep™ PCR Inhibitor Removal Kit	Fast, one step procedure for removal of PCR inhibitors such as polyphenolics, humic/fulvic acids, melanin, etc. for successful PCR and other downstream applications.	50 2x96	D6030 D6035
Plasmid DNA Purification			
Zyppy™ Plasmid Miniprep Kit	Pellet-Free™ plasmid DNA purification in less than 10 minutes. Recover up to 25 µg DNA in as low as 30 µl.	50 100 400	D4036 D4019 D4020
Zyppy™-96 Plasmid Miniprep	The fastest and simplest high-throughput method for plasmid purification.	2x96 4x96 8x96	D4041 D4042 D4043
Zyppy™ Plasmid Midiprep Kit	Pellet-Free™ plasmid DNA purification in 15 minutes in a 150 µl minimum elution volume).	25 50	D4025 D4026
ZR Plasmid MiniPrep™ Classic	Plasmid DNA purification in minutes: (alkaline lysis/spin column format for low 30 µl elution volume).	50 100 400	D4036 D4019 D4020
Genomic DNA Purification			
Quick-gDNA™ MiniPrep	Easy purification of genomic DNA from whole blood, plasma, serum, body fluids, buffy coat, lymphocytes, tissue, swabs or cultured cells in as little as 15 minutes <u>without</u> the use of Proteinase K or organic denaturants.	50 200 50 200	D3006 (uncapped) D3007 (uncapped) D3024 (capped) D3025 (capped)
ZR-96 Quick-gDNA™	Simple, high throughput (96-well) purification of DNA from whole blood, plasma, serum, body fluids, buffy coat, lymphocytes, tissue, swabs, or cultured cells in about 30 minutes.	2x96 4x96 10x96	D3010 D3011 D3012
ZR-Genomic DNA™-Tissue MiniPrep	For high quality DNA purification from <u>solid tissues</u> (e.g., tail snips, ear punches, adipose tissue, etc.), body fluids, cultured cells, buccal cells, FFPE tissues, hair, and other biological sources using Proteinase K and Fast.	50 200	D3050 D3051
Environmental DNA Purification Kits	Unique BashingBead™ technology allows isolation of DNA from samples refractory to conventional lysis procedures including tough-to-lyse tissues, soil samples, feces, plants, seeds, insects, bacteria, yeast, filamentous fungi, unicellular and filamentous algae, and protozoa		Visit website for a comprehensive list

Please visit our website to see our complete line-up of products.

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