



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Tissue & Insect DNA MidiPrep™

Catalog No. **D6115**

Highlights

- Simple method for the isolation of DNA from fresh, frozen or stored insect and arthropod specimens in as little as 20 minutes.
- The organic denaturant/proteinase K-free procedure is ideal for processing small organisms including mosquitoes, bees, lice, ticks, and *D. melanogaster*.
- State-of-the-art, ultra-high density **BashingBeads™** are fracture resistant, chemically inert and can be used with any bead mill, disrupter, or vortex that can accommodate standard 50 ml conical centrifuge tubes.
- Also, compatible with mammalian tissues, cultured cells, and whole blood.

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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 Tissue & Insect DNA MidiPrep™ (Kit Size)	D6115 (25 preps.)	Storage Temperature
ZR BashingBead™ Lysis/Filtration Tubes	25	Room Temp.
Genomic Lysis Buffer	2 x 100 ml	Room Temp.
DNA Pre-Wash Buffer*	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer	16 ml	Room Temp.
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	25	Room Temp.
Collection Tubes	50	Room Temp.
Instruction Manual	1	Room Temp.

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.

* 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

- **Format** – Bead Beating, Spin/Vacuum Filtration, and Spin Column Purification
- **Sample Sources** – Samples ($n \geq 2.5$ and ≤ 125 mg) of fresh, frozen, or stored insects including: mosquitoes, bees, lice, ticks, *D. melanogaster*, etc. Also, compatible with fresh or frozen mammalian tissues (e.g., soft tissues like liver and brain) as well as cultured cells, and whole blood.
- **DNA Purity** – High quality, humic/fulvic-free DNA is eluted with **DNA Elution Buffer** making it perfect for PCR. $A_{260}/A_{280} \geq 1.8$, $A_{260}/A_{230} \geq 2.0$
- **DNA Yield** – Expected yields can range from 1 - 5 μg DNA per mg specimen sampled. For mammalian tissues, yields are 1 - 3 μg DNA per mg of skeletal, heart and brain tissues and 3 - 5 μg DNA per mg of liver, kidney, and lung tissues. Whole blood will yield from 3 - 7 μg DNA per 100 μl .
- **DNA Size Limits** – Capable of recovering genomic DNA sized fragments from 100 bp to ≥ 40 kb. Typical fragment sizes range from 25 kb-35 kb. If present, parasitic and viral DNA will also be recovered.
- **DNA Recovery** – Typically, up to ~ 125 μg total DNA is eluted into ≥ 150 μl **DNA Elution Buffer** per sample.
- **Equipment** – Centrifuge, Vacuum Source and Manifold, Microcentrifuge, Cell Disrupter/Pulverizer w/ 50 ml Tube Adapter

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. 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.

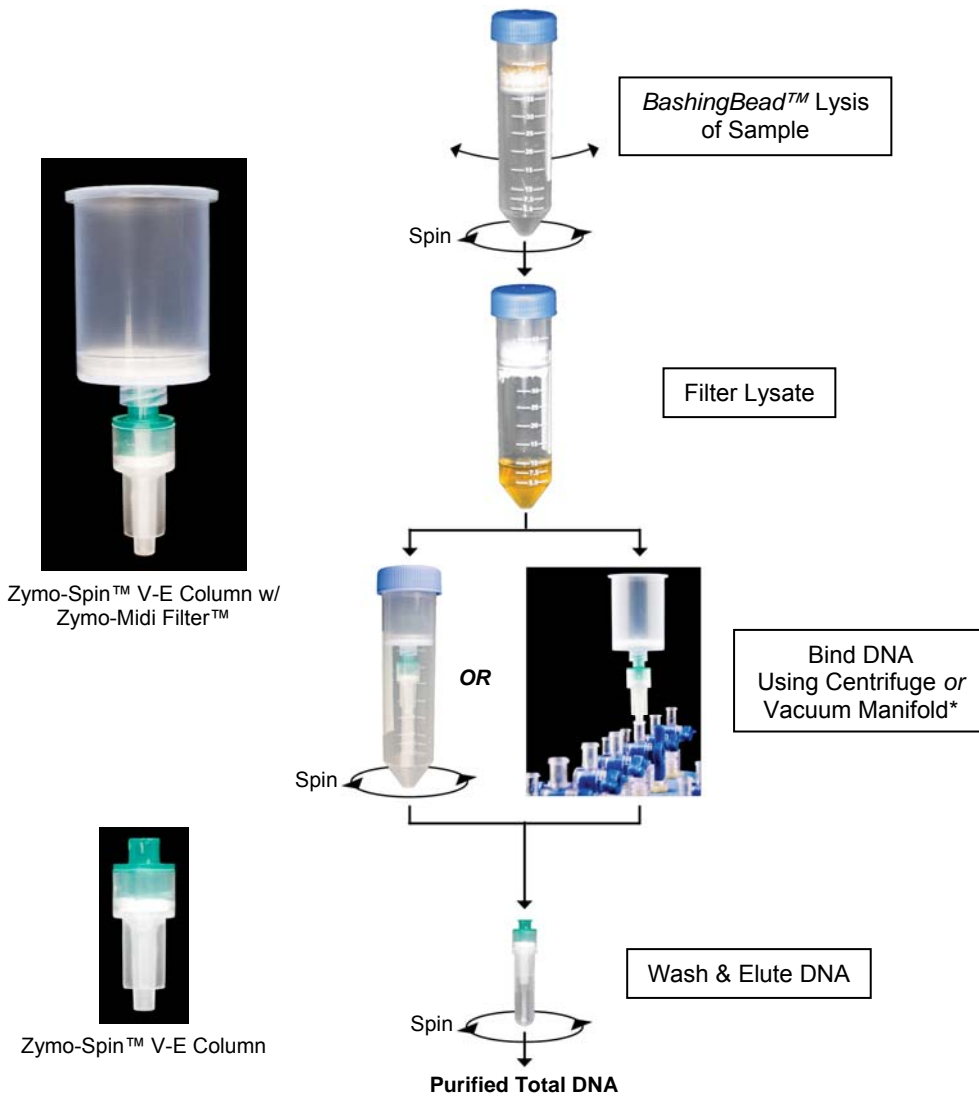
For purification of up to 25 μg DNA/prep use the **ZR Tissue & Insect DNA Kit™ (D6016)**. For high throughput purification (96-well) use the **ZR-96 Tissue & Insect DNA Kit™ (D6017)**.

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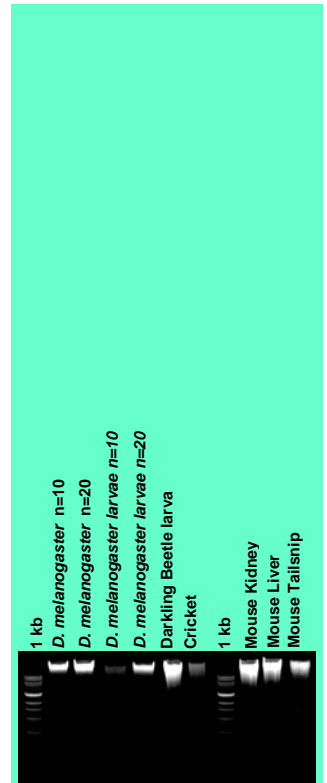
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Product Description

The **ZR Tissue & Insect DNA MidiPrep™** is designed for the simple, rapid isolation of up to 125 µg total DNA (e.g., genomic, viral, mitochondrial) from small amounts of fresh, frozen or stored insect specimens including mosquitoes, bees, lice, ticks, and *D. melanogaster*. The procedure is easy and can be completed in as little as 20 minutes: samples are added directly to a **ZR BashingBead™ Lysis/Filtration Tube** and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. The DNA is isolated and purified using our *Fast-Spin* column technology and is ideal for downstream molecular-based applications including PCR, endonuclease digestion, array, genotyping, etc. PCR inhibitors are effectively removed during the purification process. Also, the procedure is compatible with mammalian tissues, whole blood, and cultured cells. A schematic of the **ZR Tissue & Insect DNA MidiPrep™** procedure is shown below.



* Vacuum is the preferred method for DNA binding to the Zymo-Spin™ V-E column.



Comparison of DNA yields from various insect and mouse samples using the **ZR Tissue & Insect DNA Kit™**. Various amounts of sample were processed with equal volumes of eluted DNA analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. The 1 kb DNA size marker is from Zymo Research.

For **Technical Assistance**, please contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Protocol

1. Add specimens(s) to a **ZR BashingBead™ Lysis/Filtration Tube**¹. Add 6 ml **Genomic Lysis Buffer** to the sample, cap tube¹, and process.

*(To prevent the **Lysis Solution** from leaking into the bottom of the 50 ml tube, place the **ZR BashingBead™ Lysis/Filtration Tube** on its side prior to processing).*

Generally, no more than 125 mg tissue should be sampled, for larger samples will exceed the DNA binding capacity of the spin column (See **Specifications** on page 1). Up to 2 ml of whole blood or up to 3x10⁷ cells suspended in 1 ml PBS can also be sampled.

2. Secure in a bead beater fitted with a 50 ml tube holder assembly (see page 5) to process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

Processing times may be as little as 40 seconds when using high-speed cell disrupters (e.g., FastPrep®-24, Geno/Grinder®, page 5). See manufacturer's literature for operating information.

3. Centrifuge the **ZR BashingBead™ Lysis/Filtration Tube** in a centrifuge at ≥3,000 x g (5,000 x g max.) for 5 minutes.
4. Filter the entire mixture from Step 3 using a **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly mounted on a vacuum manifold^{2,3} (see diagram on page 2) with a vacuum source set at ≥600 mm Hg.

Notes:

¹ Cap tube tightly to prevent leakage.

² Be careful to avoid the pelleted material at the bottom of the tube when transferring the supernatant.

³ Alternatively, the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly can be placed in a 50 ml tube and centrifuged at 2,000 x g max. for 5 minutes. Filtration of the entire mixture will require several spins. Empty the flow through from the tube after each spin. **CAUTION: Make sure the connection between the column and filter is secure (finger tight) prior to centrifugation!**

⁴ Leave the rotor cover off the microcentrifuge if clearance with the column tops is a problem.

⁵ DNA yields can be increased by performing a second elution and pooling the eluates.

5. Disconnect the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly and transfer the **Zymo-Spin™ V-E Column** to a **Collection Tube**. Spin at 10,000 x g for 1 minute in a microcentrifuge⁴, then add 300 µl **DNA Pre-Wash Buffer** to the column and spin at 10,000 x g for 1 minute. Discard the flow through.
6. Add 400 µl **g-DNA Wash Buffer** to the column and centrifuge at 10,000 x g for 1 minute. Discard flow through and repeat wash step.
7. Transfer the **Zymo-Spin™ V-E Column** to a 1.5 ml microcentrifuge tube and add 150 µl **DNA Elution Buffer** directly to the column matrix⁵ and allow column to stand for 1 minute at room temperature. Centrifuge at 10,000 x g for 1 minute to elute the DNA.

Ultra-pure DNA is now ready for use in your experiments.

Ordering Information

Product Description	Catalog No.	Kit Size
ZR Tissue & Insect DNA Kit-5™	D6015	50 preps.
ZR Tissue & Insect DNA Kit-25™	D6016	50 preps.
ZR-96 Tissue & Insect DNA Kit™	D6017	2x96 preps.
ZR Tissue & Insect DNA MidiPrep™	D6115	25 preps.

For Individual Sale	Catalog No.	Amount
ZR BashingBead™ Lysis/Filtration Tubes (50 ml) w/ 2.0 mm Beads	S6011	25 tubes
Genomic Lysis Buffer	D3004-1-100	100 ml
DNA Pre-Wash Buffer	D3004-5-15	15 ml
g-DNA Wash Buffer	D3004-2-50	50 ml
DNA Elution Buffer	D3004-4-16	16 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25 columns/filters
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1000	1,000 tubes

Compatible Lysis Instruments...**Bullet Blender™**

NEXT >>> ADVANCE

Homogenize tissue or disrupt/lyse cells in minutes. **The Bullet Blender™**, is a vortexer (at a low setting), a cell disruptor and a tissue homogenizer (at a high setting) all in one unit. No parts contact the samples, eliminating any possibility of cross contamination.

Description	Cat No.
DX50B Bullet Blender™ Blue 50 Accommodates 9 x 50 ml tubes. Features fan cooling.	S6007-1

FastPrep®

The **FastPrep®-24 Instrument** is a unique, high-speed benchtop homogenizer that employs a powerful, proprietary technology for the rapid lysis of almost any sample in 40 seconds or less. The FastPrep® Instrument makes it possible to isolate DNA, RNA and protein from sources that are virtually impossible to lyse without the use of its rapid reciprocating motion.

Description	Cat No.
FastPrep® Instrument	S6005
BigPrep™ Attachment Accommodates 2 x 50 ml tubes.	S6005-4

**2000 Geno/Grinder®**

The **2000 Geno/Grinder Instrument** is a unique instrument that provides vigorous up-and-down grinding/pulverizing action. The Geno/Grinder instrument makes it possible to prepare plant materials such as seeds, stems, roots, leaves, and certain animal tissue. Can accommodate (2) 96-well plates/blocks for high-throughput sample processing.

Description	Cat No.
2000 Geno/Grinder Instrument	S6006
50 ml Tube Holder/Cryo Block Assembly Accommodates 12 x 50 ml tubes/block.	S6006-3



Environmental DNA & RNA Purification Products From Zymo Research

Product	Description	Kit Size	Cat No.
<i>Tough-to-Lyse and Environmental Sample DNA Isolation</i>			
ZR Soil Microbe DNA Kit™ ZR-96 Soil Microbe DNA Kit™ ZR Soil Microbe DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6001 D6002 D6101
ZR Fungal/Bacterial DNA Kit™ ZR-96 Fungal/Bacterial DNA Kit™ ZR Fungal/Bacterial DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6005 D6006 D6105
ZR Fecal DNA Kit™ ZR-96 Fecal DNA Kit™ ZR Fecal DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6010 D6011 D6110
ZR Tissue & Insect DNA Kit-5™ ZR Tissue & Insect DNA Kit-25™ ZR-96 Tissue & Insect DNA Kit™ ZR Tissue & Insect DNA MidiPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 50 preps. 2x96 preps. 25 preps.	D6015 D6016 D6017 D6115
ZR Plant/Seed DNA Kit™ ZR-96 Plant/Seed DNA Kit™ ZR Plant/Seed DNA MidiPrep™	Spin Column Format (up to 25 µg/prep.) 96-Well Format (up to 5 µg/well) Vacuum Filtration, Spin Column Format (up to 125 µg/prep.)	50 preps. 2x96 preps. 25 preps.	D6020 D6021 D6120
<i>Tough-to-Lyse and Environmental Sample RNA Isolation</i>			
ZR Fungal/Bacterial RNA MicroPrep™ ZR Fungal/Bacterial RNA MiniPrep™	Spin Column Format (up to 5 µg/prep.) Spin Column Format (up to 25 µg/prep.)	50 preps. 50 preps.	R2010 R2014
ZR Plant RNA MiniPrep™	Spin Column Format (up to 25 µg/prep.)	50 preps.	R2024
ZR Tissue & Insect RNA MicroPrep™	Spin Column Format (up to 5 µg/prep.)	50 preps.	R2030

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