ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-gDNA™ Blood MidiPrep

Catalog No. **D3074**

Highlights

- Quick method for the purification of high quality DNA from up to 3 ml *whole blood*, *plasma*, and *serum*, in less than 20 minutes using innovative *Fast-Spin* column technology. Up to 125 µg/prep.
- 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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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

***Quick-gDNA*™ Blood MidiPrep** (Kit Size)	**D3074** (25 preps.)	**Storage Temperature**
Genomic Lysis Buffer*	2x 150 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.
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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 the highest performance and reliability.

* *Recommended*: Add beta-mercaptoethanol to 0.5%(v/v) i.e., 750 µl per 150 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 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Specifications

- **Sample Sources** – Up to 3 ml (see protocol) whole blood, plasma, or serum from humans, mice, rats, etc.
- **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
- **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 125 µg total DNA is eluted into ≥150 µl **DNA Elution Buffer** or water. Human whole blood will typically yield 3-7 µg DNA per 100 µl blood sampled.
- **Product Detergent Tolerance** – ≤5% Triton X-100, ≤5% Tween-20, ≤5% Sarkosyl, ≤0.1% SDS.
- **Equipment Needed** – Centrifuge or vacuum source and manifold, microcentrifuge, vortex

Use the ***Quick-gDNA*™ Blood MiniPrep** (D3072, D3073) for the recovery of up to 25 µg/prep DNA from small samples.

For high-throughput purification (96-well, 5 µg DNA/well) use the **ZR-96 *Quick-gDNA*™ Blood** (D3075, D3076, D3077).

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.

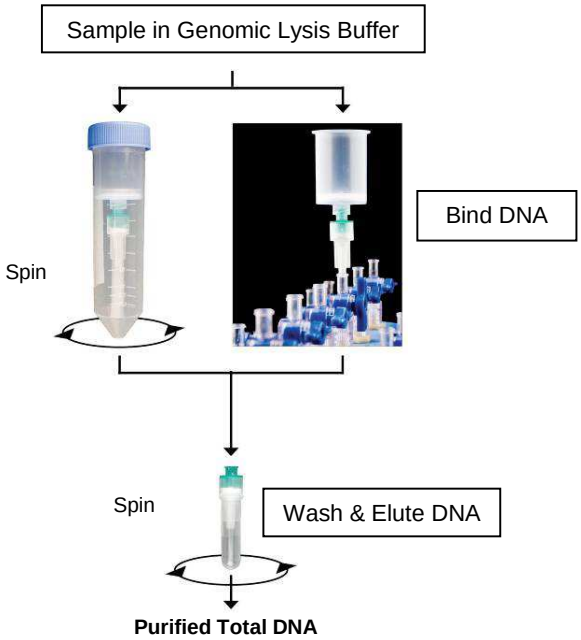
Product Description

The ***Quick-gDNA™* Blood MidiPrep** 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, and plasma.

For processing, simply add the specially formulated **Genomic Lysis Buffer** to a sample, vortex, and transfer the mixture to the supplied **Zymo-Spin™ Column w/ Zymo-Midi Filter**. 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* 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 ***Quick-gDNA*™ Blood MidiPrep** is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bisulfite conversion/methylation analysis, and other downstream applications.

Zymo-Spin™ V-E Column w/ Zymo-Midi Filter™

Zymo-Spin™ V-E Column

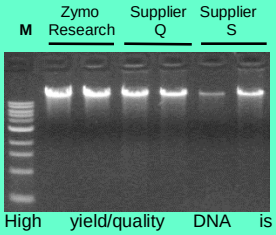


Zymo Research offers the following for rapid, precise DNA methylation detection…

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)



High yield/quality DNA is successfully isolated from porcine whole blood using the ***Quick-gDNA*™ Blood MiniPrep** D3072). Equivalent amounts (100 µl) of blood were processed without Proteinase K using the ***Quick-gDNA*™ Blood MiniPrep** 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.

Buffer Preparation

✓ *Recommended*: Add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5%(v/v) i.e., 750 µl per 150 ml.

Protocol

The following is for the purification of DNA from up to 3 ml[1] 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 12 ml of **Genomic Lysis Buffer** to 3 ml[1] 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 12 ml Genomic Lysis Buffer to *all* samples < 3 ml.

2. Transfer the mixture to a **Zymo-Spin™ V-E Column/Zymo-Midi Filter** assembly into a 50 ml tube[2]. Centrifuge at ≥1,000 *x g* (2,000 x *g* max.) for 5 minutes[3].

   Note: If using a vacuum manifold, the processing capacity is reduced to 2 ml of blood, serum, or plasma + 12 ml Genomic Lysis Buffer per prep. This filtration step may take up to twenty minutes when using vacuum.

3. 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 microcentrifuge[4] to remove residue from the column.

4. Add 300 µl **DNA Pre-Wash Buffer** to the column and spin at 10,000 x *g* for 1 minute. Discard the flow through.

5. Add 400 µl of **g-DNA Wash Buffer** to the column and centrifuge at 10,000 *x g* for one minute. Discard flow through and repeat wash step.

6. 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 matrix[5] and allow column to stand for 1 minute at room temperature. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA[6]. 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, 0-4ºC for 1-2 months, -20ºC for 6 months to a year, 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**.

For the inclusion of small DNAs from serum, add 0.3 volumes isopropanol to the mixture. (*For example, to a 15 ml mixture of serum and Genomic Lysis Buffer add 4.5 ml isopropanol.*)

Notes:

[1]Processing volumes are up to 3 ml for centrifugation and up to 2 ml for vacuum based manipulations, respectively.

[2]*Caution*: Make sure the connection between the column and filter is secure (finger tight) prior to centrifugation.

[3]Alternatively, the **Zymo-Spin™ V-E Column/Zymo-Midi Filter™** assembly can be mounted on a vacuum manifold with a vacuum source set at ≥500 mm Hg.

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

[5] 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 warmed to 60-70°C.

[6] DNA yields can be increased by performing a second elution and pooling the eluates.

Troubleshooting:

1. DNA degradation: Check for DNase contamination. All reagents supplied with the ***Quick-gDNA*™ Blood MidiPrep** 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.

4. Avian Blood: Reduce input to 50-125 µl when working with avian blood. Add 12 ml of **Genomic Lysis Buffer** to the sample. Thoroughly mix the sample by vortexing intermittently for 5 minutes. Transfer to the **Zymo-Spin™** column and proceed with step 2 of the protocol.

Ordering Information

Product Description	Cat. No.	Kit Size
***Quick-gDNA*™ Blood MicroPrep**	D3070 D3071	50 preps. 200 preps.
***Quick-gDNA*™ Blood MiniPrep**	D3072 D3073	50 preps. 200 preps.
***Quick-gDNA*™ Blood MidiPrep**	D3074	25 preps.
ZR-96 *Quick-gDNA*™ Blood	D3075 D3076 D3077	2x 96 well 4x 96 well 10x 96 well

For Individual Sale	Cat. 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	D3004-4-10	10 ml
Zymo-Spin™ V-E Columns w/ Zymo-Midi Filters™	C1021-25	25 columns/filters
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

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Popular Products From Zymo Research

Product	Description	Kit Size (Preps)	Catalog No. (Format)
	Fragment DNA Purification		
DNA Clean & Concentrator™-5	Clean and concentrate up to 5µg 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 without 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 solid tissues (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**

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