ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-gDNA™ MicroPrep

Catalog Nos. **D3020** & **D3021**

Highlights

- Quick 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).
- 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.

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

***Quick-gDNA*™ MicroPrep** (Kit Size)	**D3020** (50 Preps.)	**D3021** (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	10 ml	2 x 10 ml	Room Temp.
Zymo-Spin™ IC Columns	50	200	Room Temp.
Collection Tubes	100	400	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.

* *Recommended*: Add beta-mercaptoethanol to 0.5%(v/v) i.e., 250 µl per 50 ml *or* 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 ºC 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 total DNA is eluted into ≥10 µl (6 µl minimum) **DNA Elution Buffer** or water. Human whole blood will typically yield 1.5-3.5 µg DNA per 50 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

For high-throughput purification (96-well, 5 µg DNA/well) use the **ZR-96 *Quick-gDNA*™** (D3010, D3011, D3012).

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.

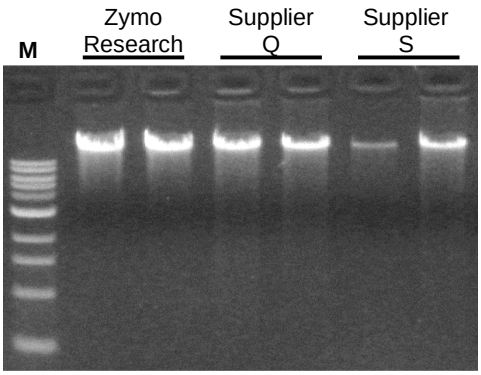
Product Description

The ***Quick-gDNA™* MicroPrep** 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* 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™* MicroPrep** is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bisulfite conversion/methylation analysis, and other downstream applications.

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 ***Quick-gDNA™* MiniPrep** (D3024). Equivalent amounts (100 µl) of blood were processed without Proteinase K using the ***Quick-gDNA™* 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).

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

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., 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 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.

For the inclusion of small DNAs from serum, add 0.3 volumes isopropanol to the mixture. (*For example, to a 1 ml mixture of serum and Genomic Lysis Buffer add 300 µl isopropanol.*)

The column capacity is ~800 µl.

1. In a 1.5 ml microcentrifuge tube, add up to 50 µl (max.) of blood, serum, or plasma to 200 µl of **Genomic Lysis Buffer**. Mix completely by vortexing 4-6 seconds, then let stand 5-10 minutes at room temperature.
2. Transfer the mixture to a **Zymo-Spin™ IC 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™ IC 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 ≥10 µl **DNA Elution Buffer** or water to the spin column. Incubate 2-5 minutes at room temperature and 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.

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.

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**.

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 µl 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 µl 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 mixture to a **Zymo-Spin™ IC 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 ~800 µl.

2. Transfer the **Zymo-Spin™ IC 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 ≥10 µl **DNA Elution Buffer** or water to the spin column. Incubate 2-5 minutes at room temperature and 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 5 mg of fresh or frozen tissue in 500 µl of* ***Genomic Lysis Buffer****.*

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™ IC 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™ IC 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 ≥10 µl **DNA Elution Buffer** or water to the spin column. Incubate 2-5 minutes at room temperature and 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 $1.0x10^6$ cells should be sampled, for larger samples will exceed the binding capacity of the spin column. See **Guidelines for Monolayer Cell Isolation** (below).

*The following procedure is designed for up to $1.0x10^6$ (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 x *g* for 5 minutes. Remove the supernatant and add 400 µl[1] of **Genomic Lysis Buffer** directly to the cell 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 by removing the medium and adding the Genomic Lysis Buffer directly to the monolayer surface.

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

2. Transfer the mixture to a **Zymo-Spin™ IC 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 ~800 µl.

3. Transfer the **Zymo-Spin™ IC 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 ≥10 µl **DNA Elution Buffer** or water to the spin column. Incubate 2-5 minutes at room temperature and 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.

[1]**Guidelines for Monolayer Cell DNA Isolation:** The above procedure is designed for the processing of $0.1\text{-}1.0x10^6$ 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. If processing more than $1.0x10^5$ cells, double the volume of **Genomic Lysis Buffer** added (i.e., 800 µl) to the sample.

Table 1: Culture Plate/Flask Growth Area (cm^2) 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 200 µl of biological liquid sample including CSF, buffy coat, body fluids (semen), and cell suspensions containing less than 1.0x10^6 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 (4:1). (e.g., add 400 µl of **Genomic Lysis Buffer** to 100 µl liquid sample). 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 10,000 *x g* for 5 minutes. Transfer up to 800 µl supernatant to the Zymo-Spin™ IC Column in Step 2.

2. Transfer the mixture to a **Zymo-Spin™ IC 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 ~800 µl.

3. Transfer the **Zymo-Spin™ IC 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 ≥10 µl **DNA Elution Buffer** or water to the spin column. Incubate 2-5 minutes at room temperature and 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 supplied with the ***Quick-gDNA*™ MicroPrep** 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 D3021	50 preps. 200 preps.
***Quick-gDNA*™ MiniPrep** w/ **uncapped** columns	D3006 D3007	50 preps. 200 preps.
***Quick-gDNA*™ MiniPrep** w/ **capped** columns	D3024 D3025	50 preps. 200 preps.
***Quick-gDNA*™ MidiPrep**	D3100	25 preps.
ZR-96 *Quick-gDNA*™	D3010 D3011 D3012	2x96 well 4x96 well 10x96 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™ IC Columns	C1004-50 C1004-250	50 250
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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