INSTRUCTION MANUAL

Quick-DNA™ Miniprep Kit

Catalog Nos. **D3024** & **D3025**

Highlights

- Quick purification of high quality DNA from whole blood, plasma, serum, body fluids, buffy coat, lymphocytes, swabs or cultured cells in less than 15 minutes using innovative Zymo-Spin™ 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.

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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*-DNA™ Miniprep Kit** (Kit Size)	**D3024** (50 Preps.)	**D3025** (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™ IIC Columns	50	200	Room Temp.
Collection Tubes	100	400	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 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, cells from culture, buccal cells, as well as a variety of biological liquids are effectively processed using this kit. Tissue already digested with Proteinase K or mechanically homogenized can also be processed.
- **Workflow Overview** – Unique lysis buffer system omits the need for Proteinase K digestion for biological fluids and cell culture samples.
- **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 25 µg total DNA is eluted into ≥50 µl (30 µl minimum) **DNA Elution Buffer** or water. Human whole blood will typically yield 3-7 µg DNA per 100 µl blood sampled. Mammalian tissues already homogenized 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 DNA isolation from biological fluids, cell cultures, and solid tissues utilizing Proteinase K, use the ***Quick*-DNA™ Miniprep Plus Kit** (D4068, D4069).

For high-throughput purification (96-well, 5 µg DNA/well), use the:

- ***Quick*-DNA™ 96 Kit** (D3010, D3011, D3012) for blood and cells.
- ***Quick*-DNA™ 96 Plus Kit** (D4070, D4071) for biological fluids, cell cultures, and solid tissues.

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.

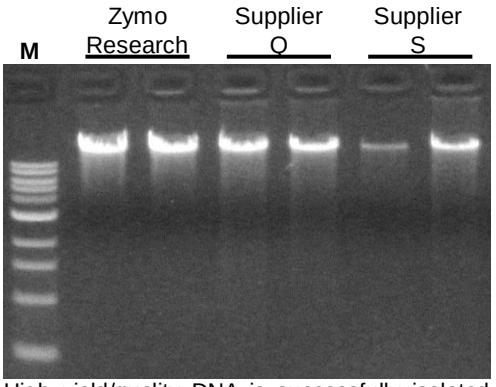
Product Description

The ***Quick*-DNA™ Miniprep 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, 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 lysis buffer system. The product features **Zymo-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*-DNA™ Miniprep Kit** 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*-DNA™ Miniprep Kit** (D3024). Equivalent amounts (100 µl) of blood were processed without Proteinase K using the ***Quick*-DNA™ Miniprep 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 routine plasmid DNA purification from *E. coli*, Zymo Research offers the **Zyppy™ Plasmid Miniprep Kit** (D4036) and the **ZymoPURE™ Midi, Maxi,** and **Gigaprep Kits** (D4200, D4202, and D4204).

Zymo Research offers the **EZ DNA Methylation-Lightning™ Kit** (D5030, D5031) for rapid, precise DNA methylation detection and a comprehensive selection of other epigenetic tools.

Looking to isolate RNA? For RNA isolation from TRIzol®, the **Direct-zol™ RNA Miniprep Kits** (R2050, R2051, R2052, R2053) offer total RNA purification without phase separation in only 7 minutes!

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 100 µl whole blood, serum or plasma (the volumes can be adjusted up to 200 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 400 µl of **Genomic Lysis Buffer** to 100 µl of blood, serum[1], 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 800 µl Genomic Lysis Buffer to 200 µl blood).*

2. Transfer the mixture[2] to a **Zymo-Spin™ IIC 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™ IIC 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[3] 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.

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.

[1] 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.*)

[2] The column capacity is 800 µl.

[3] 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 60-70ºC.

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 into a microcentrifuge tube using 500 µl of **Genomic Lysis Buffer**, vortex 4-6 seconds, and then let stand at room temperature for 5-10 minutes.

1. Transfer the mixture to a **Zymo-Spin™ IIC 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™ IIC 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[1] 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.

Solid Tissue Samples

Note: *For Proteinase K digested materials[1] (e.g., tailsnips) follow the protocol for* ***Cell Suspensions and Proteinase K Digested Samples*** *(pg. 6). Otherwise, mechanically homogenize[2] up to 25 mg of fresh or frozen tissue in 500 µl of* ***Genomic Lysis Buffer****.*

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™ IIC 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™ IIC 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.

[1] 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.

[2] For solid tissues, Proteinase K treatment or mechanical homogenization is required. For purification of up to 25 µg DNA/prep utilizing Proteinase K, use the ***Quick*-DNA™ Miniprep Plus Kit** (D4068, D4069).

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

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[1] 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] 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.

Cell Monolayer Samples

*The following procedure is designed for up to 5.0x10^6 (max.) monolayer cells[1] (roughly equal to 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).*

1. Trypsinize or manually scrape adherent cells[1] 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 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.*

[1] Generally, no more than 5.0x10^6 cells should be sampled; larger samples will exceed the binding capacity of the spin column. See **Guidelines for Monolayer Cell Isolation** (below).

2. Transfer the mixture[2,3] to a **Zymo-Spin™ IIC Column** in a **Collection Tube**. Centrifuge at 10,000 *x g* for one minute. Discard the Collection Tube with the flow through.

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

[3] The column capacity is 800 µl.

3. Transfer the Zymo-Spin™ IIC 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 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.

[4] 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.

Guidelines for Monolayer Cell DNA Isolation: The above procedure is designed for the processing of 0.1-5.0x10^6 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 1.0x10^6 cells, double the volume of **Genomic Lysis Buffer** added (i.e., 1 ml) 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[1] containing less than 5.0x10^6 cells as well as lysates derived from Proteinase K digested samples[2].

1. Add 4 volumes of **Genomic Lysis Buffer** to each volume of liquid sample (4:1). (e.g., add 800 µl of Genomic Lysis Buffer to 200 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 1 ml supernatant to the Zymo-Spin™ IIC Column in Step 2.*

2. Transfer the mixture[3] to a **Zymo-Spin™ IIC 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™ IIC 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[4] 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.

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

[2] For solid tissues, Proteinase K treatment or mechanical homogenization is required. For purification of up to 25 µg DNA/prep utilizing Proteinase K, use the ***Quick*-DNA™ Miniprep Plus Kit** (D4068, D4069).

[3] The column capacity is 800 µl.

[4] 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.

Troubleshooting:

1. DNA degradation: Check for DNase contamination. All reagents supplied with the ***Quick*-DNA™ Miniprep 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 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*-DNA™ Microprep Kit**	D3020 D3021	50 preps. 200 preps.
***Quick*-DNA™ Miniprep Kit**	D3024 D3025	50 preps. 200 preps.
***Quick*-DNA™ 96 Kit**	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™ IIC Columns	C1011-50 C1011-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000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The Beauty of Science is to Make Things Simple