ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

DNA Purification *Made Simple*™

INSTRUCTION MANUAL

Quick-DNA™ 96 Plus Kit

Catalog Nos. D4070 & D4071

Highlights

- Easy and reliable high-throughput DNA purification from any biological fluids, cultured/monolayer cells, or solid tissues.
- **Zymo-Spin™ Technology** ensures DNA is ready for all sensitive downstream applications including qPCR, DNA-sequencing, arrays, and methylation analysis.

Contents

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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™ 96 Plus Kit** (Kit Size)	**D4070** (2 x 96)	**D4071** (4 x 96)	**Storage Temperature**
Proteinase K & Storage Buffer	2 x 20 mg	4 x 20 mg	−20°C (after mixing)
BioFluid & Cell Buffer (Red)	12 ml	25 ml	Room Temp.
Solid Tissue Buffer (Blue)*	10 ml	22 ml	Room Temp.
Genomic Binding Buffer	45 ml	85 ml	Room Temp.
DNA Pre-Wash Buffer *	50 ml	2 x 50 ml	Room Temp.
g-DNA Wash Buffer	3 x 50 ml	3 x 100 ml	Room Temp.
DNA Elution Buffer	10 ml	2 x 10 ml	Room Temp.
Deep-Well Block w/ Film	2	4	Room Temp.
Zymo-Spin™ I-96-XL Plate	2	4	Room Temp.
Collection Plate	2	4	Room Temp.
Elution Plate	2	4	Room Temp.
Cover Foil	2	4	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.

* The **Solid Tissue Buffer (Blue)** and **DNA Pre-Wash Buffer** may have formed a precipitate. If this is the case, incubate at 37°C to solubilize. DO NOT MICROWAVE.

Specifications

- **Sample Sources** – See pages 2 and 3.
- **Workflow Overview** – Utilize a Proteinase K Digestion and Zymo-Spin™ Technology for effective recovery of DNA. See page 5 for more information.
- **DNA Types** – The ***Quick*-DNA™ 96 Plus Kit** will isolate total DNA including genomic, mitochondrial, plasmid, viral, parasitic, etc. from biological fluids, cultured/monolayer cells, or solid tissues. Not recommended for small cell-free DNA isolation from urine and serum/plasma (see specialized kits D3061 & D4076 respectively).
- **DNA Purity** - High quality DNA is ready for all sensitive downstream applications such as PCR, endonuclease digestion, Southern blotting, genotyping, Next-Generation Sequencing, bisulfite conversion, etc. ($A_{260}/A_{230} \geq 2.0$).
- **DNA Size** - Capable of recovering genomic and mitochondrial DNA sized fragments > 50 kb. If present, parasitic, microbial, and viral DNA will also be recovered.
- **DNA Yield** - The DNA binding capacity of each well is 5 µg. Typically, mammalian tissues yield: 1-3 µg DNA per mg skeletal, heart, lung, and brain tissues and 3-5 µg DNA per mg liver and kidney. Human whole blood will yield 3-7 µg DNA per 100 µl blood sampled.
- **Elution Volume** - DNA can be eluted into as little as 15 µl **DNA Elution Buffer** or water**.**
- **Equipment** - Water bath or heat block (55˚C), microcentrifuge, and vortex.
- **DNA Applications** – DNA isolated using the ***Quick*-DNA™ 96 Plus Kit** can be used for life-science research, genotyping, livestock breeding, veterinary research, and routine applied testing among a variety of other applications.

Sample Sources

Biological Fluids: For total DNA isolation from ≤ 50 µl of whole blood, nucleated blood, buffy coat, saliva, sputum, semen, milk, etc.

Special Considerations

- For biological fluids samples stored in DNA/RNA Shield™, see Appendix B (**pg. 8**).
- For nucleated blood samples, such as avian blood, see Appendix C (**pg. 9**).
- For blood, saliva, and cells collected onto Guthrie, FTA®, and other storage papers (cards), see Appendix F (**pg. 12**)
- For viral DNA isolation from serum/plasma samples, follow the Biological Fluids & Cells workflow. For small cell-free DNA isolation from serum/plasma samples, use the *Quick*-cfDNA™ Serum & Plasma Kit (D4076).
- To isolate cellular and/or cell-free DNA from up to 40 ml of urine samples, see the *Quick*-DNA™ Urine Kit (D3061). For cellular DNA from urine, pellet at 3,000 x *g* for 15 minutes and remove supernatant before processing using the Biological Fluids & Cells workflow.

Mammalian/Insect Cell Cultures: For total DNA isolation from ≤ 1 x 10^6 cells such as HeLa cells, HEK-293 cells, *Drosophila* cell lines, etc.

Special Considerations

- Media should be removed before processing by pelleting cells (at approximately 500 x *g* for 2 minutes depending on volume and cell type) and removing the supernatant.
- For mammalian cell samples, it is possible to reduce Proteinase K digestion time to 5 minutes at 55˚C (Step 2 on **pg. 6**).
- For cell monolayer and buccal cell preparation and collection, see Appendix A. **(pg. 7)**.
- For samples stored in DNA/RNA Shield™, see Appendix B (**pg. 8**).

Bacterial Cell Cultures: For total DNA isolation (e.g. genomic, plasmid, etc.) from ≤ 1 x 10^6 *E. coli* cells.

Special Considerations

- Media should be removed before processing by pelleting cells (at approximately 500 x *g* for 2 minutes depending on volume and cell type) and removing the supernatant.
- For *E. coli* samples, follow the Biological Fluids & Cells workflow. All other bacterial samples may be resistant to chemical lysis and Proteinase K digestion and should be processed using the ZymoBIOMICS™-96 DNA Kit (D4303. D4307. D4309).

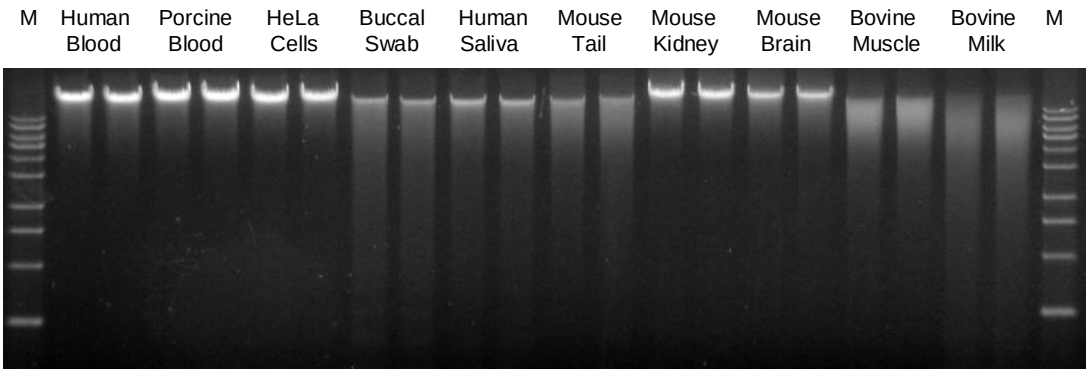
Solid Tissues: For total DNA isolation from ≤ 5 mg tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

Special Considerations

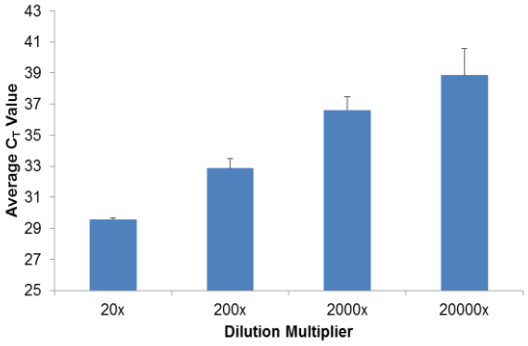
- Overnight Proteinase K digestion at 55°C is possible (Step 2, **pg. 6**).
- For solid tissue samples stored in DNA/RNA Shield™, see Appendix B (**pg. 8**).
- For hair and feather samples, see Appendix D (**pg. 10**).
- For FFPE samples, see the *Quick*-DNA™ FFPE Kit (D3067) for specialized FFPE DNA purification. See Appendix E (**pg. 11**) for an adapted protocol using the *Quick*-DNA™ 96 Plus Kit.

Product Description

The ***Quick*-DNA™ 96 Plus Kit** is the easiest method for high-throughput total DNA extraction (e.g., genomic, mitochondrial, viral) from any biological fluid, cell culture, or solid tissue sample. Innovative reagents and Zymo-Spin Technology allow for ultra-pure and concentrated genomic DNA > 50 kb to be eluted in as little as 15 µl. Zymo-Spin™ Plates ensure no buffer retention. Purified DNA is RNA-free bypassing the need for RNase A treatment and ensuring accurate quantification for applications like library preparations. Isolated DNA is suitable for immediate use in sensitive downstream applications including qPCR, DNA-seq, arrays, and methylation analysis.



High Quality DNA Obtained from a Wide Range of Biological Samples Using the *Quick*-DNA™ Miniprep Plus Kit. DNA purified using the *Quick*-DNA™ Miniprep Plus Kit is ultrapure, highly concentrated, and ready for all downstream applications. Input DNA was standardized to 300 ng and analyzed in a 1% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).



HSV-1 Viral DNA is Effectively Isolated from Plasma Using the *Quick*-DNA™ Miniprep Plus Kit. A dilution series of HSV-1 spiked into porcine plasma and extracted using the *Quick*-DNA™ Miniprep Plus Kit shows effective purification and subsequent qPCR amplification, even at a 20,000:1 dilution. The no template controls did not amplify even after 50 cycles.

The ***Quick*-DNA™ Miniprep Plus Kit** (D4068, D4069) provides processing of biological fluid, cell culture, and solid tissue samples utilizing a spin-column format.

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 Plus Kits** (R2070, R2072, R2071, R2073) offer total RNA purification without phase separation in only 7 minutes

Purification Guide

The ***Quick*-DNA™ 96 Plus Kit** facilitates high-throughput purification of DNA from any biological fluids, cultured/monolayer cells, or solid tissues by combining enzymatic and chemical extraction regimens.

Quick-DNA™ 96 Plus Kit Workflow

Biological Fluids & Cells

Biological Fluids: ≤ 50 µl
Whole blood, nucleated blood, semen, buffy coat, saliva, body fluids, milk, etc.*

Blood, saliva, and cells collected on storage paper/cards (Appendix F).

Cultured Cells: ≤ 1x10⁶
E. coli, insect, or mammalian cells (e.g. HeLa cells, buccal cells, HEK-293 cells, *Drosophila* cells, etc.).

Solid Tissues

Solid Tissues: ≤ 5 mg
Tail snips, ear punches, organ biopsies (Brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

FFPE samples (Appendix E).

Hair and feather samples (Appendix D).

BioFluid & Cell Buffer (Red)

Solid Tissue Buffer (Blue)

Proteinase K Digestion at 55˚C

Genomic Binding Buffer

Spin
Wash
Elute

Zymo-Spin™ Technology

Ultra-Pure DNA

* Viral DNA from serum or plasma samples can also be processed using this workflow. Not recommended for cell-free DNA isolation from urine, serum, or plasma samples.

For cell-free DNA isolation from up to 40 ml urine, see the ***Quick*-DNA™ Urine Kit** (D3061). For cell-free DNA isolation from up to 10 ml serum or plasma samples, see the ***Quick*-cfDNA™ Serum & Plasma Kit** (D4076).

Reagent Preparation

✓ Add 1,060 µl **Proteinase K Storage Buffer** to each **Proteinase K** (20 mg) tube prior to use. The final concentration of **Proteinase K** is ~20 mg/ml. Store at -20ºC after mixing.

Protocol

*Resuspend ≤ 1 x 10^6 mammalian or insect cell pellets using 50 µl **DNA Elution Buffer** or an isotonic buffer (e.g. PBS).*

Overnight Proteinase K digestions at 55°C are possible without affecting the integrity of the DNA.

Biological Fluids & Cells

1. Add up to 50 µl[1] sample to each well of a **Deep-Well Block** and add:

   50 µl **BioFluid & Cell Buffer (Red)**
   5 µl **Proteinase K**

2. Mix thoroughly, seal with film, and then incubate at 55ºC for 20 minutes.

3. Add 1 volume **Genomic Binding Buffer** to each well and mix thoroughly by repeated pipetting.

Example: Add 105 µl Genomic Binding Buffer to the 105 µl digested sample.

Solid Tissues

1. To tissue samples (≤ 5 mg) in a **Deep-Well Block**, add to each well a solution of:

   45 µl Water
   45 µl **Solid Tissue Buffer (Blue)**
   10 µl **Proteinase K**

2. Mix thoroughly, seal with film, and then incubate the tube at 55ºC for 1-3 hours or until tissue clarifies. Mix thoroughly before proceeding.

3. Add 2 volumes **Genomic Binding Buffer** to each well. Mix thoroughly by repeated pipetting.

Example: Add 200 µl Genomic Binding Buffer to the 100 µl mixture.

Note: To remove insoluble debris, centrifuge block at ≥ 1,000 x *g* for 5 minutes. Transfer aqueous supernatants[2] to the 96-Well Plate.

4. Transfer the lysates[2] to the wells of the **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge at ≥ 3,500 *x g* for 5 minutes[3]. Discard the flow-through.

5. Add 200 µl **DNA Pre-Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

6. Add 500 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

7. Add 200 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

8. Transfer the 96-well plate to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer** or water[4] directly on the matrix. Incubate 3 minutes at room temperature, then centrifuge at ≥ 3,500 *x g* for 5 minutes to elute the DNA[5]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

Notes:

[1] If using < 50 µl sample, increase the volume to 50 µl using **DNA Elution Buffer** or an isotonic buffer (e.g. PBS) before continuing.

[2] Avoid transferring lipid layer and pelleted cellular debris.

[3] If the lysate is still visible on top of the matrix, centrifuge for another minute or until completely cleared.

[4] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, ensure the pH is > 6.0.

[5] The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

Appendix A

Cell Monolayer Sample Preparation:

The following procedure is designed for up to 5 x 10^6 monolayer cells (dilute if necessary for proper cell counts). 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).

Trypsinize or scrape adherent cells from a culture flask or plate. Centrifuge the suspension at approximately 500 x *g* for 5 minutes. Remove the supernatant and resuspend the cell pellet in 1 ml PBS (Phosphate Buffered Saline) and then transfer suspension to a microcentrifuge tube. Centrifuge the suspension at approximately 500 x *g* for 5 minutes. Discard the supernatant and then follow the Biological Fluids & Cells workflow on Page 4.

Guidelines for Monolayer Cell DNA Isolation:

Cell numbers (growth densities) can vary between different cell types. Table 1 (below) provides an approximation of the cell numbers that can be recovered from different culture containers for "high-density" growth cells like CV1 and HeLa cells.

Table 1: Culture Plate/Flask Growth Area (cm^2) and Cell Number

Culture Container	Well /Flask Surface Area	Cell Number
96-well plate	0.32-0.6 cm^2	4-5 x 10^4
24-well plate	2 cm^2	1-3 x 10^5
12-well plate	4 cm^2	4-5 x 10^5
6-well plate	9.5 cm^2	0.5-1 x 10^6
T25 Culture Flask	25 cm^2	2-3 x 10^6
T75 Culture Flask	75 cm^2	0.6-1 x 10^7
T175 Culture Flask	175 cm^2	2-3 x 10^7

Buccal Cells and Swabs:

Buccal cells can be isolated using a rinse- or swab-based isolation method.

A. Rinse Method: Vigorously rinse mouth with 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. Then follow from Step 1 of the Biological Fluids & Cells workflow on Page 4.

B. Swab Isolation Method: Thoroughly rinse mouth out with water 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 deep well block using a solution of 100 µl of **BioFluid & Cell Buffer (Red)** and 100 µl TE or another isotonic solution. Add 10 µl of **Proteinase K,** mix thoroughly, and incubate at 55ºC for 20 minutes. Then follow from Step 3 of the Biological Fluids & Cells workflow on Page 4 (dilute or remove sample if needed to reduce the cell count).

Appendix B

Samples in DNA/RNA Shield™:

DNA/RNA Shield™ ensures nucleic acid stability during sample storage/transport at ambient temperatures. There is no need for refrigeration or specialized equipment. DNA/RNA Shield™ effectively lyses cells and inactivates nucleases and infectious agents (virus), and it is compatible with various collection and storage devices (vacutainers, swabs, nasal, buccal, fecal etc.).

DNA/RNA Shield™ purchased separately (R1100 or R1200).

Biological Fluids

1. Add 5 µl of Proteinase K to 100 µl of the sample/shield mixture prepared according to the DNA/RNA Shield™ specifications.
2. Mix thoroughly or vortex 10-15 seconds and then incubate at room temperature for 20 minutes.
3. Continue from Step 3 of the Biological Fluids & Cells Workflow (**pg. 6**).

Solid Tissues

1. To each 100 µl sample prepared according the DNA/RNA Shield™ specifications[1], add 50 µl of the **Solid Tissue Buffer (Blue)** and 10 µl of Proteinase K to the lysate.
2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at 55ºC 1 – 3 hours[2].

   Note: Overnight digestion at 55°C is possible and will increase the effectiveness of digestion and DNA recoveries.
3. To remove insoluble debris, centrifuge at ≥ 3,500 x g for 5 minutes. Transfer aqueous supernatant to a clean 96-well block.
4. Add 1 volume **Genomic Binding Buffer** to the digested sample. Mix thoroughly or vortex 10-15 seconds.
5. Continue from Step 4 of the main protocol (**pg. 6**).

Notes:

[1] Tissue samples can be mechanically homogenized for optimal extraction efficiency.

[2] Optimal incubation times may vary with tissue type and homogenization method.

Appendix C

Nucleated Blood Samples

1. Add up to 5 µl of nucleated blood to the following in a Deep-Well Block:

BioFluid & Cell Buffer (Red)	50 µl
Proteinase K	5 µl
DNA Elution Buffer (or TE Solution)	to make 105 µl total

2. Mix thoroughly by pipetting up and down, seal with film, and then incubate at 55ºC for 20 minutes.

Note: The sample may not be completely homogenous before digesting.

3. Add 1 volume of **Genomic Binding Buffer** to each well and mix thoroughly by repeat pipetting. Ensure the sample is homogenous before continuing.

Note: It may be necessary to pipette up and down many times to ensure the sample is homogenous. Vortexing will also help ensure the mixture is homogenous.

4. Transfer the lysates to the wells of the **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge at ≥ 3,500 *x g* for 5 minutes[1]. Discard the flow-through.

5. Add 200 µl **DNA Pre-Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

6. Add 500 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

7. Add 200 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

8. Transfer the 96-well plate to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer** or water[2] directly on the matrix. Incubate 3 minutes at room temperature, then centrifuge at ≥ 3,500 *x g* for 5 minutes to elute the DNA[3]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

Notes:

[1] If the lysate is still visible on top of the matrix, centrifuge for another minute or until completely cleared.

[2] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[3] The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

Appendix D

Hair and Feather Samples:

1. Freshly prepared DTT (dithiolthreitol) (not provided) needs to be added to each well containing sample (≤ 5 mg) as follows:

Water	40 µl
Solid Tissue Buffer (Blue)	45 µl
DTT (1 M)	5 µl
Proteinase K	10 µl

2. Mix thoroughly, seal with film, and then incubate the tube at 55°C for 1-3 hours.

   Note: Overnight digestions are possible without affecting the integrity of the DNA.

3. Add 200 µl **Genomic Binding Buffer** to the wells and mix thoroughly by repeat pipetting. Centrifuge at ≥ 1,000 *x g* for 5 minutes to pellet insoluble debris.

4. Transfer the mixture (supernatant) to **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

5. Add 200 µl **DNA Pre-Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

6. Add 500 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

7. Add 200 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

8. Transfer the 96-well plate to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer** or water[1] directly on the matrix. Incubate 3 minutes at room temperature, then centrifuge at ≥ 3,500 *x g* for 5 minutes to elute the DNA[2]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

Notes:

[1] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[2] The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Alternatively, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

Notes:

The ***Quick*-DNA™ FFPE Kit** (D3067) is specialized for DNA purification from FFPE samples.

[1] It is possible to store samples at -80°C at this point for later use.

[2] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[3] The total yield can be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

Appendix E

FFPE Samples:

Deparaffinize FFPE Samples:

1. Remove or trim as much paraffin from the sample(s) as possible (≤ 5 mg).
2. Transfer samples to each well of a Deep-Well Block. Add 750 µl xylene (not provided) to the samples.
3. Mix and incubate samples at room temperature for 1 hour with gentle rocking.
4. Centrifuge for 5 minutes at 1,000 *x g* and remove the xylene from the sample. Repeat steps 2-4.
5. Wash with 1 ml ethanol (100%), mix briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 1,000 *x g* for 5 minutes, discard the supernatant, and repeat.
6. Wash with 1 ml ethanol (95%), mix briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 1,000 *x g* for 5 minutes, discard the supernatant, and repeat.
7. Wash with 1 ml ethanol (75%), mix briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 1,000 *x g* for 5 minutes, discard the supernatant, and repeat.
8. Wash with 1 ml ddiH_2O, mix briefly, and incubate for 5 minutes with gentle rocking. Centrifuge at ≥ 1,000 *x g* for 5 minutes and remove the water from the sample[1].

DNA Extraction:

1. Prepare the Proteinase K Digestion to the deparaffinized samples in each well as follows:

Water	45 µl
Solid Tissue Buffer (Blue)	45 µl
Proteinase K	10 µl

2. Mix thoroughly, seal with film, and incubate the tube at 55°C for 12-16 hours.
3. Incubate the block at 94°C for 20 minutes.
4. Add 6 volumes **Genomic Binding Buffer** to the wells and mix thoroughly by repeat pipetting. Centrifuge at ≥ 1,000 *x g* for 5 minutes to pellet insoluble debris.
9. Transfer the mixture (supernatant) to **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.
10. Add 200 µl **DNA Pre-Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.
11. Add 500 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.
12. Add 200 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.
13. Transfer the 96-well plate to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer** or water[2] directly on the matrix. Incubate 3 minutes at room temperature, then centrifuge at ≥ 3,500 *x g* for 5 minutes to elute the DNA[3]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20°C for future use.

Appendix F

Samples Collected onto Storage Papers/Cards:

Rapid purification of inhibitor-free, PCR-quality DNA from blood, saliva, and cells collected onto Guthrie, FTA®, and other storage papers (cards). The procedure is easy; card punches are added directly to ZR-96 BashingBead Lysis Rack (2.0 mm) and thoroughly homogenized by bead beating (e.g. 2000 GenoGrinder®, or similar). Following Proteinase K digestion, the DNA is purified using innovative Zymo-Spin™ Technology. Eluted DNA is ideal for PCR, genotyping, etc.

ZR-96 BashingBead Lysis Rack (2.0 mm) (S6002-96-2) and Lysis Solution (D6001-3) purchased separately.

1. Add card samples (punches) to a **ZR-96 BashingBead™ Lysis Rack (2.0 mm)**[1]. Add 400 µl **Lysis Solution**[2] to each tube.

2. Secure in a 96-well block/plate bead beater (e.g., 2010 GenoGrinder®) and process samples. Optimization of processing time/speed will be necessary for complete sample lysis.

   Note: Processing times may be as little as one minute when using high-speed cell disrupters (e.g., 2000 GenoGrinder® , page 5). See manufacturer's literature for operating information.

3. Centrifuge the **ZR-96 BashingBead™ Lysis Rack (2.0 mm)** at 3,000 *x g* (5,000 *x g* max) for 5 minutes.

4. To the lysate in the **ZR-96 BashingBead™ Lysis Rack (2.0 mm)**, add:

Proteinase K	40 µl
Solid Tissue Buffer (Blue)	360 µl

5. Mix and then incubate the tubes at 55˚C for 10-15 minutes.

6. Centrifuge the **ZR-96 BashingBead™ Lysis Rack (2.0 mm)** for 5 minutes. Transfer 400 µl supernatant to each well in a **Deep-Well Block**.

7. Add 800 µl **Genomic Binding Buffer** to each well and mix thoroughly.

8. Transfer 600 µl of the mixture to a **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge at ≥ 3,500 *x g* for 5 minutes.

9. Discard the flow through from the Collection Plate and repeat Step 8.

10. Add 200 µl **DNA Pre-Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

11. Add 500 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

12. Add 200 µl **g-DNA Wash Buffer** to each well. Centrifuge at ≥ 3,500 *x g* for 5 minutes. Discard the flow-through.

13. Transfer the 96-well plate to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer** or water[3] directly on the matrix. Incubate 3 minutes at room temperature, then centrifuge at ≥ 3,500 *x g* for 5 minutes to elute the DNA[4]. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

[1] **ZR-96 BashingBead Lysis Rack (2.0 mm)** (Catalog No. S6002-96-2)

[2] **Lysis Solution** (Catalog No. D6001-3-40 & D6001-3-150)

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[4] The total yield can be improved by eluting the DNA with 60-70 ˚C **DNA Elution Buffer**. Also, loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.

GenoGrinder® is a registered trademark of Spex SamplePrep®, LLC

Quick-DNA™ 96 Plus Kit
Quick Protocol
Catalog Nos. D4070 & D4071

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

Biological Fluids & Cells Protocol

Biological Fluids: ≤ 50 µl
Total DNA from whole blood, buffy coat, saliva, sputum, semen, etc. See the Instruction Manual page 2 for other samples and special considerations.

Cultured Cells: ≤ 1x10^6 cells
Total DNA from *E. coli*, insect, or mammalian cells (e.g. HeLa cells, buccal cells, HEK-293 cells, etc.). See the Instruction Manual page 2 for special considerations and sample preparation information.

Note: *Pellet cells and discard supernatant. **Resuspend** ≤ 1 x 10^6 cell pellets using 50 µl **DNA Elution Buffer** or an isotonic buffer (e.g PBS).*

**Add 1,060 µl of Storage Buffer to each 20 mg tube of Proteinase K. Store at -20°C.*

1. Add up to 50 µl sample to each well of a **Deep-Well Block** and add:
   50 µl **BioFluid & Cell Buffer (Red)**
   5 µl **Proteinase K**

   Note: *For inputs < 50 µl biological fluid, increase the volume to 50 µl with DNA Elution Buffer or an isotonic buffer before continuing.*

2. Mix thoroughly, seal with film, and then incubate at 55ºC for 20 minutes.

3. Add 1 volume **Genomic Binding Buffer** to the digested sample. Mix thoroughly.

   Example: *Add 105 µl Genomic Binding Buffer to the 105 µl digested sample.*

4. Transfer the lysates to the wells of the **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge (≥ 3,500 *x g*) for 5 minutes. Discard the flow-through.

5. Add 200 µl **DNA Pre-Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

6. Add 500 µl **g-DNA Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

7. Add 200 µl **g-DNA Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

8. To elute the DNA, transfer to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer**, incubate 3 minutes, and then centrifuge for 5 minutes.

DNA Purification Made Simple™

For the full Instruction Manual, visit
http://www.zymoresearch.com/m/D4070

Ver. 1.1.0

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Quick-DNA™ 96 Plus Kit
Quick Protocol
Catalog Nos. D4070 & D4071

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

Solid Tissues Protocol

Solid Tissues: ≤ 5 mg
Total DNA from tail snips, ear punches, organ biopsies (brain, liver, heart, kidney, muscle, stomach, bladder, intestine, etc.).

For special sample types including FFPE, hair and feather, see the Instruction Manual page 3.

**Add 1,060 µl of Storage Buffer to each 20 mg tube of Proteinase K. Store at -20°C.*

1. To tissue samples (≤ 5 mg) in a **Deep-Well Block**, add a solution of:
   45 µl Water
   45 µl **Solid Tissue Buffer (Blue)**
   10 µl **Proteinase K**

2. Mix thoroughly, seal with film, and then incubate at 55ºC for 1-3 hours or until tissue clarifies. Mix thoroughly.

3. Add 2 volumes **Genomic Binding Buffer** to each well. Mix thoroughly.

   Example: *Add 200 µl Genomic Binding Buffer to the 100 µl mixture.*

   Note: *To remove insoluble debris, pellet by centrifugation at ≥ 1,000 x g for 5 minutes. Transfer aqueous supernatants to the 96-Well Plate.*

4. Transfer the lysates to the wells of the **Zymo-Spin™ I-96-XL Plate** on a **Collection Plate**. Centrifuge (≥ 3,500 *x g*) for 5 minutes. Discard the flow-through.

5. Add 200 µl **DNA Pre-Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

6. Add 500 µl **g-DNA Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

7. Add 200 µl **g-DNA Wash Buffer** to each well and centrifuge for 5 minutes. Discard the flow-through.

8. To elute the DNA, transfer to an **Elution Plate**. Add ≥ 15 µl **DNA Elution Buffer**, incubate 3 minutes, and then centrifuge for 5 minutes.

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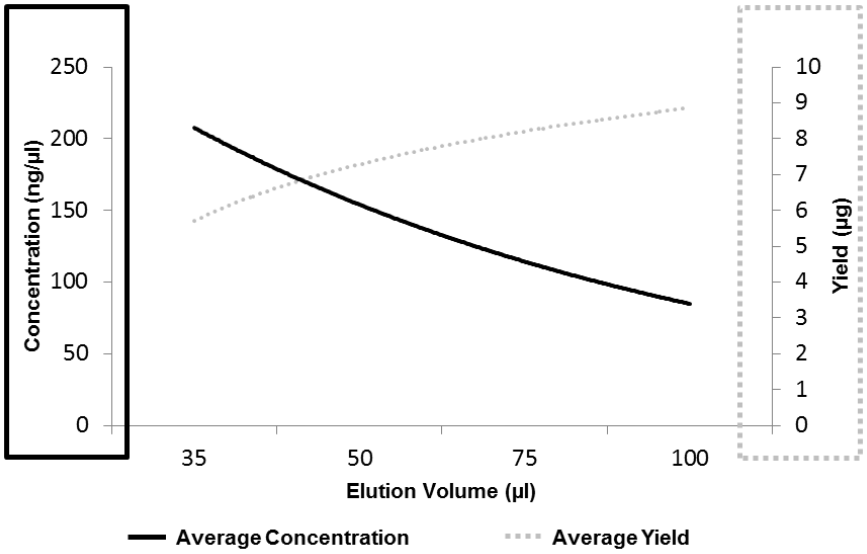
Troubleshooting:

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

Problem	Possible Causes and Suggested Solutions
DNA Elution Guide	*Identifying Proper Elution Volume*  **The Relationship Between Elution Volume, DNA Yield, and DNA Concentration Using Porcine Whole Blood (Column Format)**. Using a smaller elution volume results in higher concentrations of DNA samples, but with reduced yields. Using a larger elution volume results in higher DNA yields, but at a reduced concentration. Choose an elution volume that best fits your individual application. *Increasing DNA Yields* • The total yield may be improved by eluting the DNA with DNA Elution Buffer pre-heated to 60-70°C. • Loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again can also increase total yield.
DNA Degradation	*DNase Contamination* • Check pipets, pipet tips, microcentrifuge tubes, etc. for DNase contamination and exercise the appropriate precautions during the DNA purification procedure. All reagents and components supplied with the ***Quick*-DNA™ 96 Plus Kit** are DNase-free. However, DNase contamination can result during the processing of some samples. • If water is used to elute the DNA, ensure that DNase-free water is used. • Certain samples are more prone to degradation as a result of the conditions used for storage and transport (e.g. FFPE Tissue).

Low DNA Yield

Incomplete Debris Removal

- For solid tissue samples, ensure lysate is centrifuged after digestion to pellet insoluble debris. Transfer the supernatant while avoiding any pelleted debris or lipid layer.

Incomplete Lysis/Digestion

- Ensure Proteinase K digestions are performed at 55ºC as indicated. It is possible to extend digestion times if samples are high in protein.
- Mix samples longer after the addition of Genomic Binding Buffer to ensure that the lysate is homogenous.

Tissue Input

- For low DNA-containing tissues (e.g. muscle, etc.) using larger inputs will increase yields (≤ 5mg).
- If the lysate does not pass through the column or is extremely viscous, use less input material. Too much tissue can cause cellular debris to overload the column and leech salts into the DNA eluate.

Elution Procedures

- Ensure the DNA Elution Buffer hydrates the matrix for 5 minutes at room temperature before centrifugation.
- To increase yields, heat the DNA Elution Buffer to 60-70˚C before use. You can also load the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again.

Procedural Errors

- Ensure the proper digestion buffer is used. See the Purification Guide on page 3.
- Ensure the correct volume of Genomic Binding Buffer is used. For plasma and serum samples, use 3 volumes of Genomic Binding Buffer. See the Purification Guide on page 3 and the Protocol on page 4.

Low DNA Performance

Procedural Errors

- The tip of a well is contaminated with wash buffer flow through. Avoid tilting the plate during the wash steps and ensure the tip does not touch the flow through. Empty the collection plate when instructed.
- Insufficient centrifugation: Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the final wash step by 5 minutes to ensure complete wash buffer removal.

Low DNA Performance
Continued

Tissue Input

- Make sure the lysate has passed completely through the matrix before proceeding to the wash steps.
- If the lysate does not pass through the column or is extremely viscous, use less input material. Too much tissue can cause cellular debris to overload the column and leech salts into the DNA eluate.

RNA in Eluate

- All reagents and components supplied with the ***Quick*-DNA™ 96 Plus Kit** are designed for RNA removal. Typically if RNA is in the eluate, too much tissue/sample was used.
- Ensure the proper amount of Genomic Binding Buffer and corresponding digestion buffer is used. See the Purification Guide on page 3.
- Ensure Proteinase K digestions are performed at 55°C as indicated.
- For applications sensitive to trace amounts of RNA, additional RNA removal may be necessary using an RNase A treatment.

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-DNA™ Miniprep Plus Kit**	D4068 D4069	50 preps. 200 preps.
***Quick*-DNA™ Microprep Plus Kit**	D4074	50 preps.
***Quick*-DNA™ 96 Plus Kit**	D4070 D4071	2 x 96 preps. 4 x 96 preps.

For Individual Sale	Catalog No.	Amount
Proteinase K & Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
BioFluid & Cell Buffer (Red)	D4068-1-12 D4068-1-25	12 ml 25 ml
Solid Tissue Buffer (Blue)	D4068-2-10 D4068-2-22	10 ml 22 ml
Genomic Binding Buffer	D4068-3-45 D4068-3-85	45 ml 85 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 D3004-2-200	50 ml 100 ml 200 ml
DNA Elution Buffer	D3004-4-4 D3004-4-10 D3004-4-50	4 ml 10 ml 50 ml
Zymo-Spin™ I-96-XL Plates	C2010	2 plates
Collection Plates	C2002	2 plates
Elution Plates	C2003	2 plates
Deep-Well Blocks w/ Film	P1002-2	2 blocks