ZYMO RESEARCH

DNA Purification Made Simple™

Quick-DNA™ MagBead Plus Kit

Rapid high-throughput method for DNA isolation from any Sample.

Highlights

- Any Sample Type: Extract DNA from any sample type including biological fluids, blood, saliva, solid tissues, swabs and more.
- Ultra-Pure: Highest DNA yield and purity with RNA removal technology ready for all sensitive downstream applications such as qPCR, DNA sequencing, arrays, and methylation analysis.
- Automation Ready: Automation friendly workflow ready to be implemented on all open platform automated liquid handlers and bead moving devices.

Catalog Numbers:
D4081, D4082 (Patent Pending)

Scan with your smart-phone camera to view the online protocol/video.

Table of Contents

Product Contents

Quick-DNA™ MagBead Plus Kit	D4081 (1 x 96 Preps.)	D4082 (4 x 96 Preps.)	Storage Temperature
Proteinase K & Storage Buffer[1]	2 x 20 mg	8 x 20 mg	-20°C (after mixing)
Biofluid & Solid Tissue Buffer	25 ml	100 ml	Room Temp.
Quick-DNA™ MagBinding Buffer	150 ml	3 x 150 ml	Room Temp.
DNA Pre-Wash Buffer[2]	2 x 50 ml	250 ml	Room Temp.
g-DNA Wash Buffer	500 ml	3 x 500 ml	Room Temp.
DNA Elution Buffer	50 ml	3 x 50 ml	Room Temp.
MagBinding Beads	8 ml	24 ml	Room Temp.

[1] Prior to use, reconstitute the lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20ºC.
[2] 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 Types** – Any cells, solid tissue, whole blood, saliva, biological fluids, buccal, swabs, stool, microbiome samples, samples stored in DNA/RNA Shield™, *etc.*

- **DNA Purity** – High quality DNA is ready for all sensitive downstream applications such as long read sequencing, PCR, endonuclease digestion, Southern blotting, genotyping, Next-Generation Sequencing, bisulfite conversion, *etc*. ($A_{260}/A_{230} \geq 1.8$).

- **DNA Yield** – The DNA binding capacity is 10 µg per 50 µl MagBinding Beads used.

- **DNA Size** – Capable of recovering genomic and mitochondrial DNA sized fragments up to 150 kb. If present, plasmid, parasitic, microbial, and viral DNA will also be recovered.

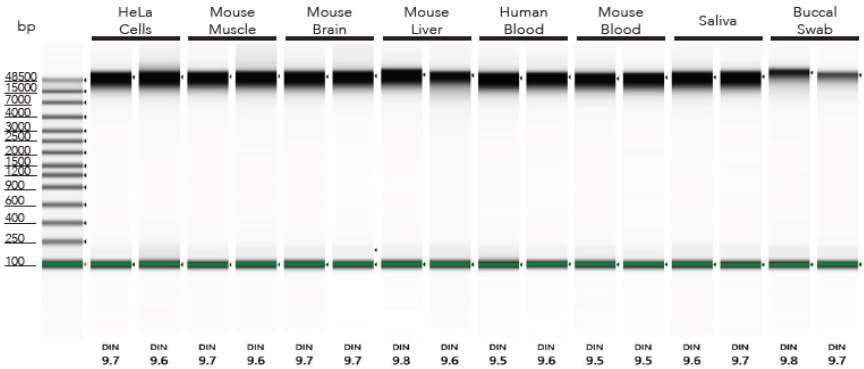
- **Elution Volume** – 50 µl **DNA Elution Buffer** per 33 µl MagBinding Beads.

- **Equipment** – Magnetic rack, shaker and/or rotator, automated liquid handler (optional)

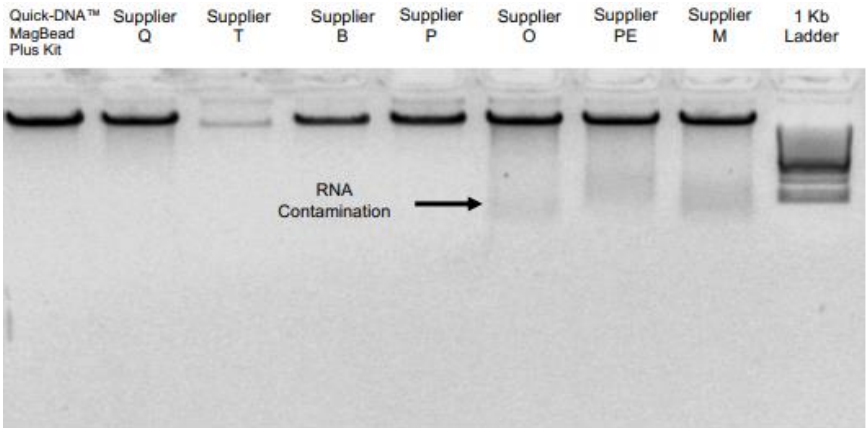
- **Automation** – For assistance with automating/scripting this workflow onto your device, contact one of our automation experts at automation@zyomresearch.com.

Product Description

The ***Quick*-DNA™ MagBead 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 Research's unique system allow for a simple Bind, Wash, & Elute procedure that is unmatched in providing ultra-pure and high yielding DNA (up to 150 kb). Isolated DNA is ready for immediate use in sensitive downstream applications including DNA sequencing (NGS and long read), qPCR, arrays, and methylation analysis.

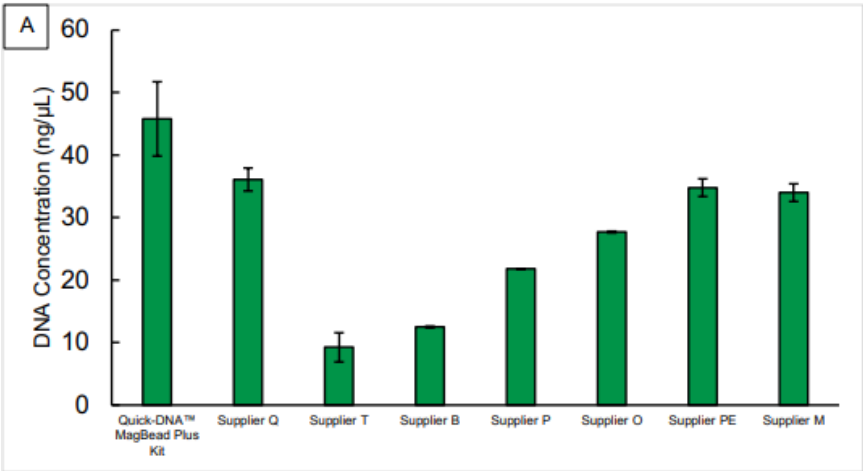


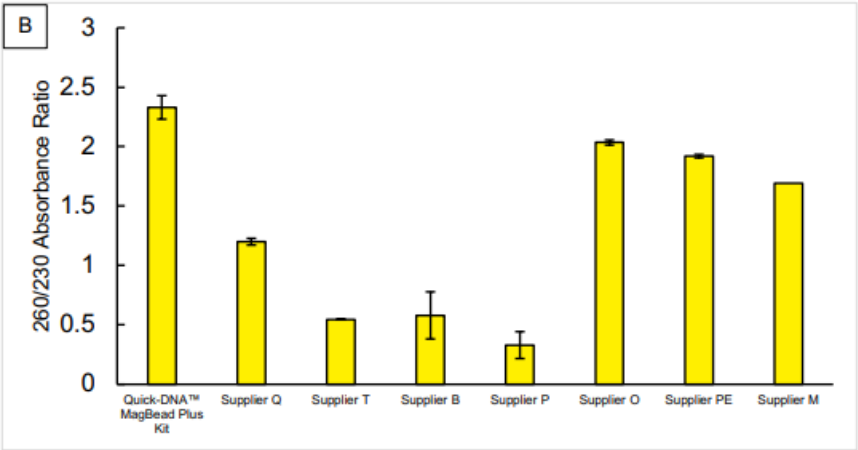
High Quality DNA From Any Sample Type. 10^6 Mammalian HeLa cells, 25 mg mouse muscle, brain, and liver, 200 µl human blood, 200 µl mouse blood, 200 µl human saliva, and buccal swabs stored in DNA/RNA Shield (R1100-50) were extracted using the *Quick*-DNA™ Magbead Plus Kit (n=2). DNA is of high molecular weight size (>60 kb). Quality was assessed using Agilent 2200 TapeStation®.



High DNA Yields With RNA Removal Technology. 200 µl human blood was processed using the Quick-DNA™ MagBead Plus Kit compared to various competitor MagBead purification kits (n=2). Input DNA was analyzed in a 1% (w/v) TAE/agarose/EtBr gel (shown above). The gel electrophoresis was prematurely paused to check for RNA contamination.

Product Description (cont.)

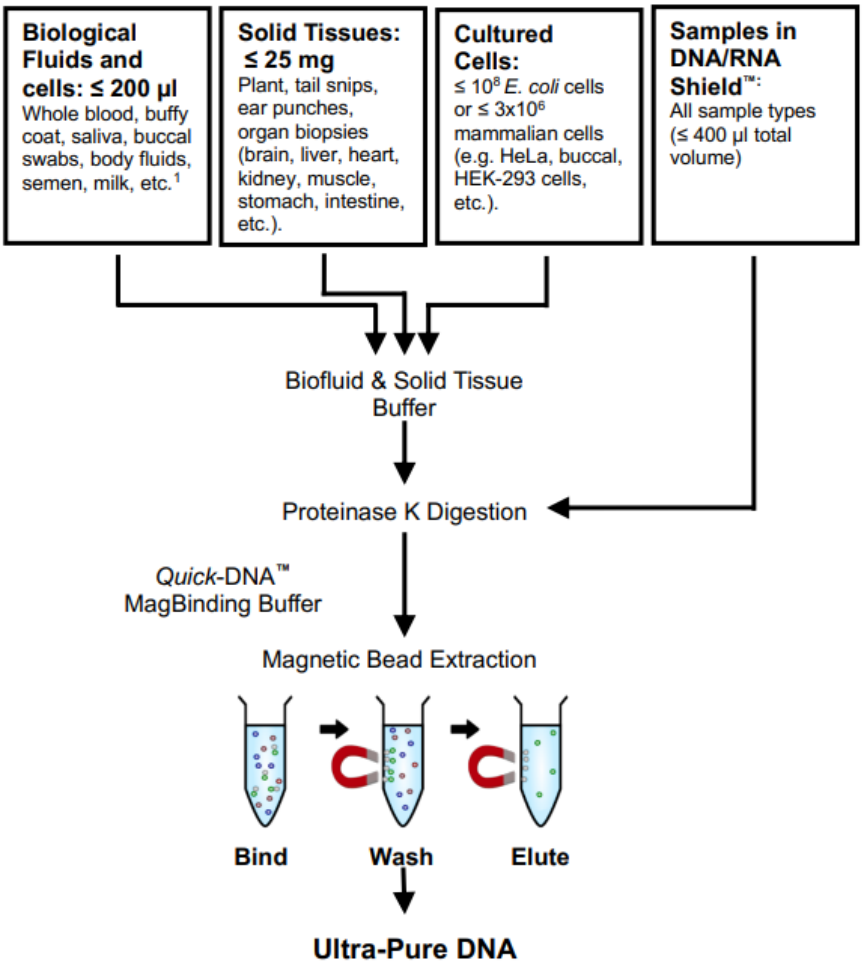




Ultra-Pure. 200 µl human blood was processed using the Quick-DNA™ MagBead Plus kit against various competitor kits and eluted with 100 µl (n=2). Zymo Research had higher or comparable DNA recovery (ng/µl; A) and consistently higher purities (A260/230: >1.8; B). Absorbance A260/230 and total DNA recovery were quantified by NanoDrop™ 2000.

Purification Guide

The ***Quick*-DNA™ MagBead Plus Kit** facilitates rapid and efficient purification of genomic DNA from any sample type by combining enzymatic and chemical extraction regimens.



[1] 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.

Protocol

Reagent Preparation

- ✓ Add 1,040 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.

- ✓ Mix the **MagBinding Beads** until the beads are completely resuspended before use.

Sample Preparation

All steps should be performed at room temperature (20-30ºC) unless specified.

Biological Fluids & Cells (Whole Blood, Saliva, *etc.*) ≤ 200 µl

1. Add 200 µl (equal volume) **Biofluid & Solid Tissue Buffer** to 200 µl liquid sample[1] and mix thoroughly.

2. Add 20 µl **Proteinase K** and pipette mix 5 times. Incubate at room temperature (20-30 °C) for 20 minutes.

3. Proceed to DNA Purification (Page 7).

Solid Tissue (Ear/Tail Snips, Liver, Plants, *etc.*) ≤ 25 mg

1. Add ≤ 25 mg solid tissue to 95 µl **DNA Elution Buffer**[2], 95 µl **Biofluid & Solid Tissue Buffer**, and 10 µl **Proteinase K**.

2. Pipette mix 5 times and incubate at 55ºC for 1-3 hours or until tissue solubilizes.

3. Centrifuge the sample at ≥ 10,000 x g with a microcentrifuge for 1 minute to pellet the debris.

4. Remove up to 400 µl of the supernatant while avoiding debris and transfer it to a new tube.

5. Proceed to DNA Purification (Page 7).

Samples in DNA/RNA Shield™ ≤ 400 µl or 25 mg

1. Add 20 µl **Proteinase K** to 400 µl sample in **DNA/RNA Shield**™[2] and mix well. Incubate at room temperature (20-30ºC) for 30 minutes.

2. Proceed to DNA Purification (Page 7).

Environmental (Plant / Fungi) ≤ 50 mg

1. Add up to 50 mg plant material and 750 µl **DNA/RNA Shield**™ to a bead beating tube[3] and mechanically homogenize your sample at maximum speed for 1 minute.

2. Centrifuge the sample at 10,000 x g for 1 minute to pellet the debris and transfer up to 400 µl of lysate into a new tube.

3. Proceed to DNA Purification (Page 7).

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[1] If using < 200 µl sample, increase the volume to 200 µl using TE Buffer or an isotonic buffer before continuing.
[2] **DNA/RNA Shield**™ (R1100-50) is sold separately.
[3] **ZR BashingBead**™ **Lysis Tubes (2.0 mm)** (S6003-50) are sold separately.

DNA Purification

1. Add 400 µl (equal volume) **Quick-DNA™ MagBinding Buffer** to 400 µl digested sample.
2. Pipette mix the solution.
3. Add 33 µl of **MagBinding Beads**[1] to each sample.
4. Mix the sample by pipette mixing or by shaker[2] for 10 minutes.
5. Transfer the sample to the magnetic stand[3] until beads have separated from the solution, then remove[4] and discard the supernatant. Transfer the sample off the magnetic stand.
6. Add 500 µl ***Quick*-DNA™ MagBinding Buffer**.
7. Mix the sample by pipette mixing or by shaker[2] for 10 minutes.
8. Transfer the sample to the magnetic stand until beads have separated from the solution, then remove[4] and discard the supernatant. Transfer the sample off the magnetic stand.
9. Add 500 µl **DNA Pre-Wash Buffer.**
10. Mix the sample by pipette mixing (~10 times) or by shaker[2] for 1 minute.
11. Transfer the sample to the magnetic stand until beads have separated from the solution, then remove[4] and discard the supernatant. Transfer the sample off the magnetic stand.
12. Add 900 µl **g-DNA Wash Buffer**[5].
13. Mix the sample by pipette mixing (~10 times) or by shaker[2] for 1 minute[6].
14. Transfer the sample to the magnetic stand until beads have separated from the solution, and then remove[4] and discard the supernatant. Transfer the sample off the magnetic stand.
15. Repeat steps 12-14 two more times.
16. To dry the beads, transfer the sample to a heated element and incubate at 55ºC for 10 minutes. If no heating element is available, air dry for 20 minutes[7].
17. Add 50 µl of **DNA Elution Buffer**[8] to each sample.
18. Mix via shaker at room temperature for 5 minutes.
19. Transfer the sample to the magnetic stand until beads have separated from solution, then transfer the eluted DNA to a new tube (plate). The eluted DNA can be used immediately or stored at ≤-20ºC.

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[1] MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.
[2] Shaking speeds can be different for each shaker. Shaker should be fast enough to completely resuspending the beads (1100 – 1500 rpm).
[3] Magnetic stand (manual processing) or strong-field 96-well magnetic stand (i.e., **ZR-96 MagStand**, P1005).
[4] Some beads will adhere to the sides of the well. When removing supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.
[5] If high speed shaker plates are used, dispense 500 µl **g-DNA Wash Buffer**.
[6] To avoid salt carryover, you may transfer your samples to a new tube / plate between each wash step.
[7] Over drying the beads may result in lower DNA recovery. Beads will change in appearance from glossy black when still wet to a matte black/brown when fully dry.
[8] **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.

Appendices

Appendix A: Ezymatic Digestion of Microbes

Enzymatic lysis of cells walls (e.g. Lysozyme, Zymolyase) from microbes is necessary to effectively isolate high molecular weight DNA from microbes.

Fluids (Whole Blood, Saliva, Water DNA/RNA Shield, Feces) ≤ 200 µl

1. Add 100 µl (equal volume) **DNA/RNA Shield™ (2x Concentrate)**[1] to up to 100 µl sample and pipette mix 10 times[2].
2. Incubate at room temperature (20-30°C) on a tube rotator or shaker for 5 minutes. Proceed to Microbial Lysis.

Cells and Solids (Cultured Cells, Feces, Soil, etc.) ≤ 100 mg or 10^8 bacterial cells

1. Resuspend up to 100 mg of sample or up to 10^8 cells with 200 µl **DNA/RNA Shield™**[3] in a microcentrifuge tube and pipette mix well.
2. Incubate at room temperature (20-30°C) on a tube rotator for 5 minutes. Proceed to Microbial Lysis.

Microbial Lysis

1. Centrifuge at 5,000 x g in a microcentrifuge for 1 minute to pellet the sample. Transfer the supernatant (~180 µl) in a **new** microcentrifuge tube. **Save both the supernatant and pellet.**
2. Add 100 µl PBS (user supplied) to sample pellet and pipette mix until pellet is visibly resuspended.
3. Centrifuge at 5,000 x g for 1 minute to pellet the sample. Combine the supernatant with the original sample supernatant (total ~280 µl) from the previous step.
4. Add 1 ml PBS (user supplied) to the new pellet and mix until pellet is visibly resuspended.
5. Centrifuge at 5,000 x g in a microcentrifuge for 1 minute to pellet the sample and discard the supernatant.
6. Add 100 µl TE Buffer and 25 µl lysozyme[4] (100 mg/ml; user supplied) to the pellet.
7. Pipette mix until pellet is visibly resuspended, then incubate at 55°C for 30 minutes.
8. Combine the saved supernatant (~280 µl) with the 125 µl digested sample.
9. Add 20 µl 10% SDS (user provided) and 10 µl **Proteinase K**. Briefly pipette mix and incubate at 55°C for 10 minutes.
10. Centrifuge 5,000 x g in a microcentrifuge for 1 minute to pellet residual debris. Transfer the supernatant to a new microcentrifuge tube.
11. Add 800 µl (2 volumes) **Quick-DNA™ MagBinding Buffer** to the sample and mix well.
12. Proceed to step 3 of DNA Purification on Page 7.

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[1] **DNA/RNA Shield™ (2X Concentrate)** (R1200-25) is sold separately.
[2] If sample is already resuspended in DNA/RNA Shield™, add 100 µl **DNA/RNA Shield™**.
[3] **DNA/RNA Shield™** (R1100-50) is sold separately.
[4] Lysozyme (100 mg/mL) is available through Sigma-Aldrich (L2879-1G)

Appendix B: Cell Monolayer Sample Preparation

The following procedure is designed for up to 3 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 PB and then transfer suspension to a new 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 6.

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 the Biological Fluids & Cells workflow on Page 6.

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 96-well plate using an isotonic solution and follow the Biological Fluids & Cells workflow on Page 6.

Appendix C: Nucleated Blood Samples

1. Add up to 5 µl of nucleated blood to the following in a microcentrifuge tube:

Biofluid & Solid Tissue Buffer	50 µl
Proteinase K	5 µl
DNA Elution Buffer (or TE Solution)	45 µl

2. Mix thoroughly by pipetting up and down. Then incubate the tube at 55°C for 30 minutes.

3. Add 100 µl (equal volume) of **Biofluid & Solid Tissue Buffer** to the tube and mix thoroughly by pipetting up and down. Ensure the sample is homogenous before continuing.

4. Proceed with DNA Purification on Page 7.

Troubleshooting

Problem	Possible Causes and Solutions
Low DNA Yield/Quality	**Binding Time.** Make sure to incubate on rotator or shaker for 10 minutes after the *Quick-DNA* MagBinding Buffer has been added to the sample. Incubation for longer periods of time may help to increase yield **Amount of MagBinding Beads.** The volume of beads used can be increased to 50 µl and eluted in 100 µl to increase the maximum binding capacity and accommodate samples of high biomass. 33 µl is the recommended starting point and can bind up to 10 µg (sample type dependent). **Proteinase K Digestion.** The optimal time is largely sample dependent. 30 minutes is recommended for liquids whereas solid tissues may be incubated overnight for complete digestion. This will maximize yields but increases protocol time. **Resuspension of Beads.** The MagBinding Beads settle quickly. Ensure complete resuspension before use by thoroughly shaking and/or vortexing the bottle. **Increase mixing cycles and/or speed.** Pipette mixing of the sample is crucial for some key steps (after adding Proteinase K and after adding MagBinding Beads) to ensure sufficient resuspension. Combined tip mixing and shaking at each step is recommended for optimal DNA yields and purities. **Prolonged Time or Increased Temperature.** Over-drying beads will result in severely reduced yields. To remove residual liquid, incubating at 55˚C for 10 minutes is a good starting point but can depend on specific plate dimensions and heater used.

Low DNA Yield or Quality	**Low Concentration.** If the final concentration of your extracted DNA is too low, use 15 µl MagBinding Beads and 30 µl DNA Elution Buffer when processing similar samples in future. **Incomplete Elution.** The recommended minimum elution volume is 1.5X ratio of the MagBinding Beads used (Ex. 50 µl beads to 75 µl elution). Using more volume ensures better surface coverage whereas using less volume can result in severely reduced yields and purities. **Temperature Conditions.** Incubating the elution step at ≥ 55˚C during the minutes of shaking time may increase final yield.
Low Purity	**New Tube Transfer.** It is crucial to transfer the g-DNA Wash / MagBead mixture to a new 1.5 mL microcentrifuge tube or 96-well plate during both wash steps. This prevents salt carryover which can lower purities. **Resuspension of Beads.** The MagBinding Beads settle out of solution quickly, so it is important to pre-mix the beads by pipette mixing to ensure full homogeneity before additional mixing via rotator or shaker. **Insufficient Mixing.** It is important to properly mix the DNA Elution Buffer when added to the MagBinding Beads. Inefficient mixing can result in lower purities.
Low Molecular Weight DNA	**Vortex and Shaking at High Speeds.** Mixing the sample using rigorous mixing parameters (e.g., vortex and shaking at high speeds) may cause shearing of the DNA, resulting in lower size recovery. Mixing via a rotator is recommended for higher size recovery.

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA™ MagBead Plus Kit	D4081-E D4082-E	1 x 96 Preps. 4 x 96 Preps.

Individual Kit Components	Catalog No.	Amount
Proteinase K & Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Biofluid & Solid Tissue Buffer	D4081-3-25 D4081-3-100	25 ml 100 ml
***Quick*-DNA™ MagBinding Buffer**	D4077-1-150 D4077-1-250	150 ml 250 ml
DNA Pre-Wash Buffer	D3004-5-15 D3004-5-30 D3004-5-50 D3004-5-250	15 ml 30 ml 50 ml 250 ml
g-DNA Wash Buffer	D3004-2-50 D3004-2-100 D3004-2-200 D3004-2-250 D3004-2-500	50 ml 100 ml 200 ml 250 ml 500 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10 D3004-4-16 D3004-4-50	1 ml 4 ml 10 ml 16 ml 50 ml
MagBinding Beads	D4100-4-3 D4100-4-8 D4100-4-12 D4100-4-16 D4100-4-24	3 ml 8 ml 12 ml 16 ml 24 ml

Complete Your DNA Methylation Workflow

✓ **Rapid Method for Complete Bisulfite Conversion of DNA**

EZ DNA Methylation Kits	Size	Catalog No.
EZ DNA Methylation-Lightning Kit	50 Rxns. 200 Rxns.	D5030 D5031
EZ-96 DNA Methylation-Lightning Kit	2x96 Rxns. (Deep-Well) 2x96 Rxns. (Shallow-Well)	D5032 D5033
EZ DNA Methylation-Lightning Automation Kit	96 Rxns.	D5049
EZ-96 DNA Methylation Lightning MagPrep	4 X 96 Rxns. 8 X 96 Rxns.	D5046 D5047

✓ **Innovative Solutions for Next Generation Sequencing**

Library Prep Kits	Size	Catalog No.
Zymo-Seq WGBS Library Kit	24 Preps.	D5465
Pico Methyl-Seq Library Prep Kit	10 Preps. 25 Preps.	D5455 D5456
Zymo-Seq RRBS Library Kit	24 Preps. 48 Preps.	D5460 D5461

✓ **Optimal Amplification of Bisulfite-Treated DNA**

ZymoTaq Polymerase	Size	Catalog No.
ZymoTaq Premix	50 Rxns. 200 Rxns.	E2003 E2004
ZymoTaq DNA Polymerase	50 Rxns. 200 Rxns.	E2001 E2002
ZymoTaq qPCR Premix	50 Rxns. 200 Rxns.	E2054 E2055

✓ **Industry Leading Tools for Assessing Your DNA Methylation Workflow**

DNA Methylation Standards	Size	Catalog No.
Human Methylated & Non-methylated DNA Set	5 µg/20 µl	D5014
Universal Methylated DNA Standard	Human Mouse	D5011 D5012
Bisulfite-Converted Universal Methylated Human DNA Standard	1 µg/50 µl	D5015
Human Methylated & Non-Methyated (WGA) DNA Set	5 µg/20 µl	D5013

Notes

Notes

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