



ZYMO RESEARCH

DNA  
Purification  
*Made Simple™*

## Quick-DNA™ HMW MagBead Kit

High molecular weight DNA isolation for any downstream application

### Highlights

- High Molecular Weight DNA: Extract high molecular weight DNA up to 150 kb from mammalian sample types (e.g. biological fluids, cultured cells, etc.).
- Ultra-Pure: Highest DNA yield and purity equipped with RNA removal technology.
- Third-Generation Sequencing Ready: Optimized for long read sequencing (including PacBio™ and Oxford Nanopore™ sequencing).

Catalog Numbers:  
D6060



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



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# Product Contents

<b>Quick-DNA™ HMW MagBead Kit</b>	<b>D6060 (96 Preps.)</b>	<b>Storage Temperature</b>
Proteinase K & Storage Buffer <sup>1</sup>	2 x 20 mg	-20°C (after mixing)
Biofluid & Solid Tissue Buffer	25 ml	Room Temp.
Quick-DNA™ MagBinding Buffer	150 ml	Room Temp.
DNA Pre-Wash Buffer <sup>2</sup>	50 ml	Room Temp.
g-DNA Wash Buffer	400 ml	Room Temp.
DNA Elution Buffer	50 ml	Room Temp.
MagBinding Beads	8 ml	Room Temp.

<sup>1</sup> Prior to use, reconstitute the lyophilized **Proteinase K** with 1040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20°C.

<sup>2</sup> A precipitate may have formed in the **DNA Pre-Wash Buffer** during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37°C for 30 minutes and mix by inversion. **DO NOT MICROWAVE.**

# Specifications

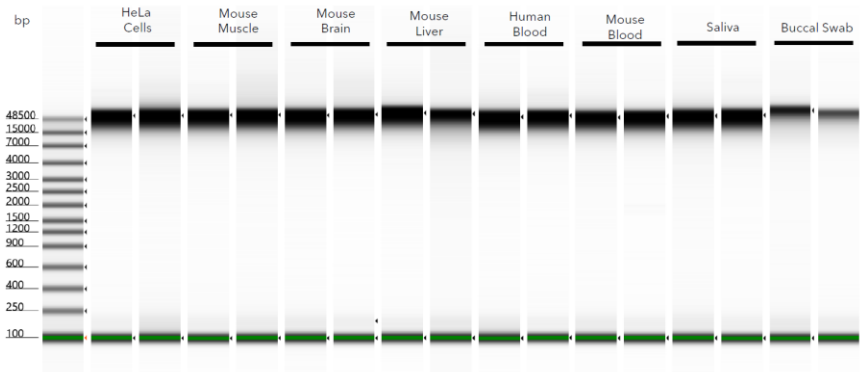
- **Sample Types** – Mammalian cells, solid tissue, whole blood, saliva, biological fluids, buccal, swabs, samples stored in DNA/RNA Shield™, *etc.*
- **DNA Purity** – High quality DNA is ready for all sensitive downstream applications such as long read sequencing, PCR, endonuclease digestion, Southern blotting, genotyping, Next-Generation Sequencing, bisulfite conversion, *etc.* ( $A_{260}/A_{280}$  and  $A_{260}/A_{230} \geq 1.8$ ).<sup>1</sup>
- **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@zymoresearch.com](mailto:automation@zymoresearch.com).

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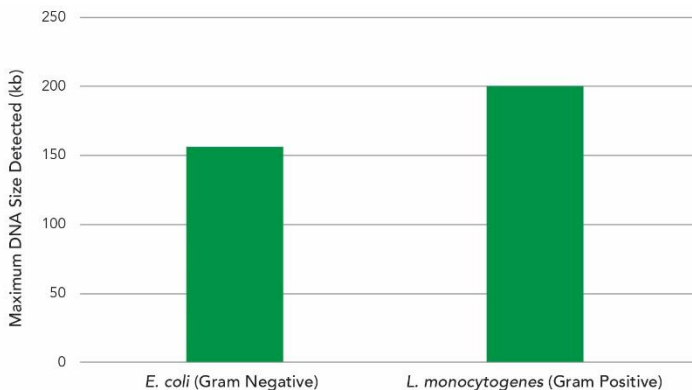
<sup>1</sup>For microbiome or metagenomic applications, we recommend using the **ZymoBIOMICS® DNA** product line.

# Product Description

The **Quick-DNA™ HMW MagBead 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.<sup>1</sup> 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 of >150 kb. Isolated DNA is ready for immediate use in sensitive downstream applications including long read (PacBio™, Oxford Nanopore™, etc.) and NGS sequencing, qPCR, arrays, and methylation analysis.



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

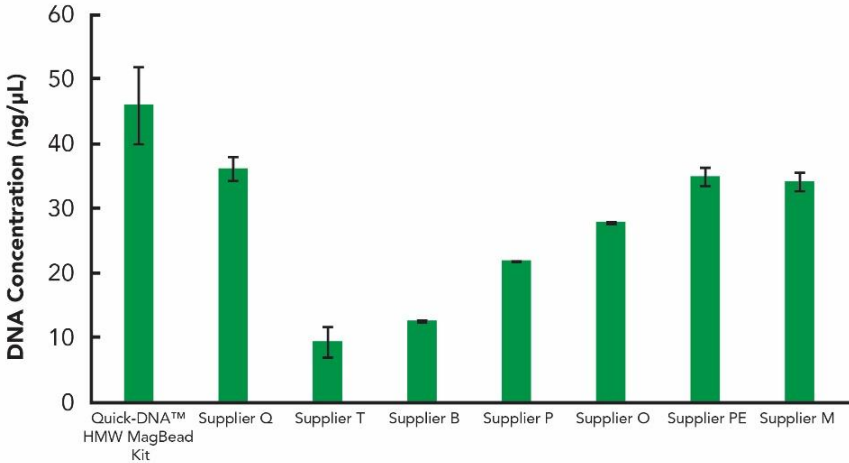


**High Molecular Weight DNA.** Cultured *E. coli* (~10<sup>9</sup> cells) and cultured *L. monocytogenes* (~10<sup>8</sup> cells) were input into the Quick-DNA™ HMW MagBead kit (n=2). Length of the highest detected peak were recorded and averaged for each sample. DNA size was analyzed using Agilent's FEMTO Pulse™ system.

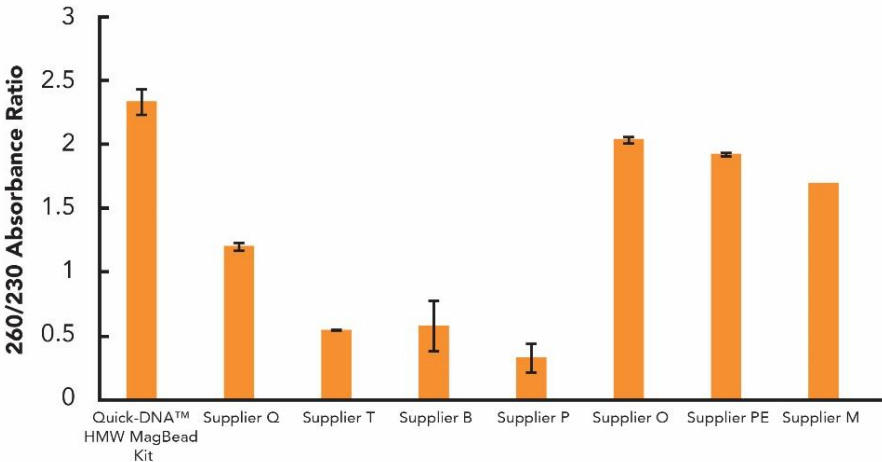
<sup>1</sup>For microbiome or metagenomic applications, we recommend using the ZymoBIOMICS® DNA product line.

# Product Description (cont.)

**A)**



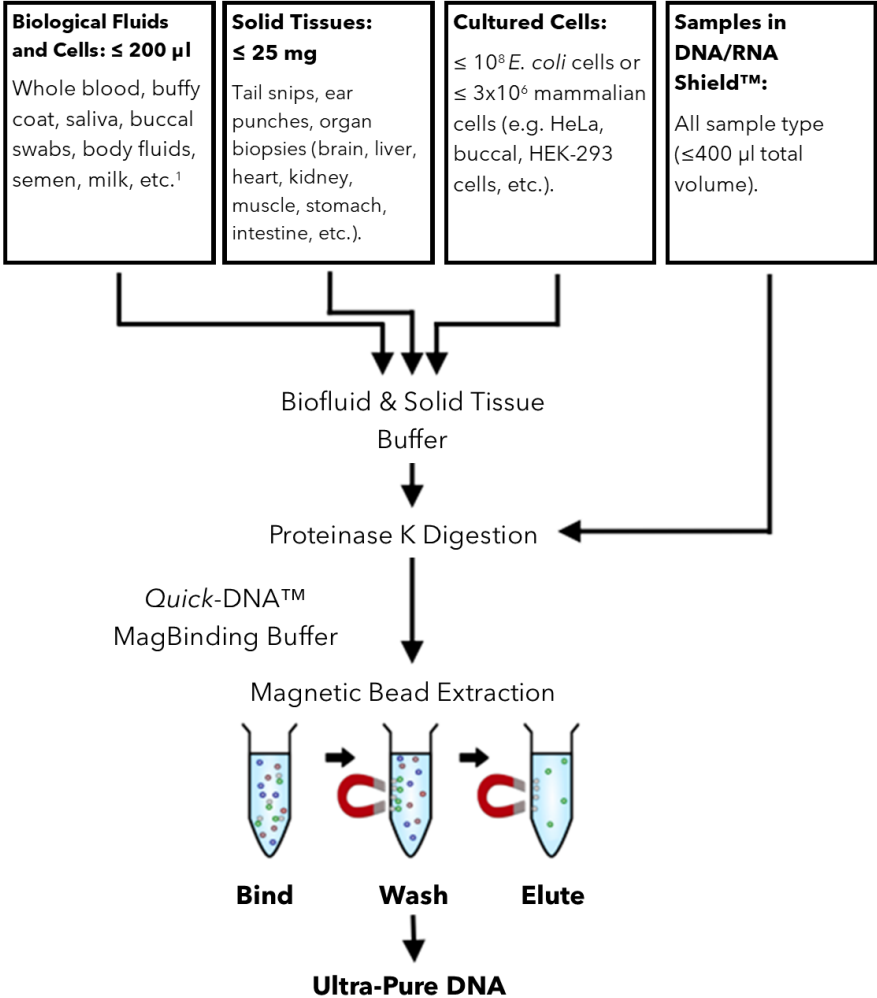
**B)**



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

# Purification Guide

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



<sup>1</sup> 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

The protocol consists of: (I) Reagent Preparation, (II) Sample Preparation and (III) DNA Purification.

## (I) Reagent Preparation

- ✓ Add 1,040  $\mu$ l **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 -20 °C after mixing.
- ✓ Mix the **MagBinding Beads** until the beads are completely resuspended before use.

## (II) Sample Preparation

All steps should be performed at room temperature (20 – 30°C) unless specified.  
Wide bore tips can be used to prevent shearing of DNA.

### **Biological Fluids & Cells** (Whole Blood, Saliva, etc.) $\leq$ 200 $\mu$ l

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*Resuspend cultured cells using 200  $\mu$ l of DNA Elution Buffer or isotonic buffer (e.g. PBS).*

1. Add 200  $\mu$ l (equal volume) **Biofluid & Solid Tissue Buffer** to 200  $\mu$ l liquid sample<sup>1</sup> and mix thoroughly.
2. Add 20  $\mu$ l **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, etc.) $\leq$ 25 mg

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1. Add  $\leq$  25 mg solid tissue to 95  $\mu$ l **DNA Elution Buffer**, 95  $\mu$ l **Biofluid & Solid Tissue Buffer**, and 10  $\mu$ l **Proteinase K**.
2. Pipette mix 5 times and incubate at 55°C for 1-3 hours or until tissue solubilizes.
3. Centrifuge the sample at  $\geq$ 10,000 x g with a microcentrifuge for 1 minute to pellet the debris.
4. Remove up to 400  $\mu$ l 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™** $\leq$ 400 $\mu$ l

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1. Add 20  $\mu$ l **Proteinase K** to 400  $\mu$ l sample in **DNA/RNA Shield™**<sup>2</sup> and mix well. Incubate at room temperature (20 – 30°C) for 30 minutes.
2. Proceed to DNA Purification (Page 7).

### **Cultured Bacterial and Fungal Cells** $\leq$ 10<sup>8</sup> bacteria/fungal cells

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1. Refer to the Appendix for sample preparation procedure.

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<sup>1</sup> If using < 200  $\mu$ l sample, increase the volume to 200  $\mu$ l using TE Buffer or an isotonic buffer before continuing.  
<sup>2</sup> **DNA/RNA Shield™** (R1100-50) is sold separately.

### (III) DNA Purification

1. Add 400  $\mu$ l (equal volume) of **Quick-DNA™ MagBinding Buffer** to the 400  $\mu$ l sample.
2. Pipette mix the solution.
3. Add 33  $\mu$ l of **MagBinding Beads**<sup>1</sup> to each sample.
4. Pipette mix 5 times and place the samples on a rotator or shaker<sup>2</sup> for 10 minutes.
5. Transfer the sample to the magnetic stand<sup>3</sup> until beads have separated from the solution, then remove<sup>4</sup> and discard the supernatant. Transfer the sample off the magnetic stand.
6. Add 500  $\mu$ l of **Quick-DNA™ MagBinding Buffer**.
7. Pipette mix to resuspend the beads (~5 times) and then place samples on a rotator or shaker for 5 minutes.
8. Transfer the sample to the magnetic stand until beads have separated from the solution, then remove<sup>4</sup> and discard the supernatant. Transfer the sample off the magnetic stand.<sup>5</sup>
9. Add 500  $\mu$ l of **DNA Pre-Wash Buffer**.
10. Pipette mix to resuspend the beads (~10 times).
11. Transfer the sample to the magnetic stand until beads have separated from the solution, then remove<sup>4</sup> and discard the supernatant. Transfer the sample off the magnetic stand.
12. Add 900  $\mu$ l of **g-DNA Wash Buffer** and pipette mix to resuspend the beads (~10 times).
13. **Transfer all liquid into a new microcentrifuge tube or 96-well plate.**
14. Transfer the sample to the magnetic stand until beads have separated from the solution, and then remove<sup>4</sup> 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 55°C for 10 minutes. If no heating element is available, air dry for 20 minutes.<sup>6</sup>
17. Add 50  $\mu$ l of **DNA Elution Buffer**<sup>7</sup> to each sample.
18. Pipette mix 20 times and incubate at room temperature for 5 minutes or mix via shaker for 5 minutes at room temperature (20 – 30°C).
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  $\leq -20^{\circ}\text{C}$ .

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<sup>1</sup> **MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.

<sup>2</sup> Shaking speeds can be different for each shaker. Shaker should be fast enough to completely resuspend the beads (1100 – 1500 rpm).

<sup>3</sup> Magnetic stand (manual processing) or strong-field 96-well magnetic stand (i.e., **ZR-96 MagStand**, P1005).

<sup>4</sup> 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.

<sup>5</sup> Optional RNase treatment can be performed after this step. See Appendix.

<sup>6</sup> 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.

<sup>7</sup> **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

## Optional RNase Treatment

This modification is necessary for downstream applications that are sensitive to trace amount of RNA. This step is not required for most long read sequencing applications (e.g., PacBio™, Oxford Nanopore™).

1. Continue after step 8 of the DNA Purification protocol on page 7.
2. Add 100 µl of **DNA Elution Buffer** to the beads.
3. Pipette mix to resuspend the beads (~5 times).
4. Add 2 µl of RNase A (1 mg/ml) to the sample.
5. Pipette mix to resuspend the beads (~5 times).
6. Incubate at room temperature (20 – 25°C) for 20 minutes.
7. Add 500 µl of **Quick-DNA™ MagBinding Buffer**.
8. Pipette mix to resuspend the beads (~5 times) and then place samples on a rotator or shaker for 10 minutes.
9. Transfer the sample to the magnetic stand until beads have separated from the solution, then remove and discard the supernatant. Transfer the sample off the magnetic stand.
10. Continue to step 9 of the DNA Purification protocol on page 7.

## Cultured Bacterial and Fungal Cells

Enzymatic digestion of cell walls (e.g. Lysozyme, Zymolyase Ultra, Zymolyase) is not advised for mixed microbial community analysis. For validated unbiased metagenomic measurements, ZymoBIOMICS® DNA Kits<sup>1</sup> featuring ZR BashingBead™ Lysis Tubes is recommend.

Lysozyme primarily digests the cells walls of gram-positive bacteria; however, not all bacteria are susceptible to Lysozyme. Therefore, other lytic enzymes (e.g., Mutanolysin, Lysostaphin) may be required for certain bacterial species.

The cell walls of fungi are not susceptible to Lysozyme digestion, so yeast lytic enzymes, such as Zymolyase, Zymolyase Ultra, Chitinase, or Lyticase must be used to degrade the cell walls of yeast and fungi.

Cell wall susceptibility to enzymatic digestion can vary with culture age. Fresh, early log-phase cells are recommended. Optimal harvest time may require further optimization. Older cells or cells in stationary phase may require more enzyme or longer incubation with the enzyme.

1. Centrifuge at 5,000  $\times g$  in a microcentrifuge for 1 minute to pellet the sample. Carefully decant and discard the supernatant.
2. Add 100  $\mu\text{l}$  TE Buffer and 25  $\mu\text{l}$  lysozyme<sup>2</sup> (100 mg/ml; user supplied) to the pellet. Optional: Add 0.28  $\mu\text{l}$  of beta-mercaptoethanol (user supplied) to the pellet.<sup>3</sup>
3. Pipette mix until the pellet is visibly resuspended, then incubate at 55°C for 30 minutes.<sup>4</sup>
4. Add 125  $\mu\text{l}$  of **Biofluid & Solid Tissue Buffer** and mix well.
5. (Optional) Centrifuge at 5,000  $\times g$  in a microcentrifuge for 1 minute to pellet residual debris. Transfer the supernatant to a new microcentrifuge tube.
6. Add 500  $\mu\text{l}$  (2 volumes) of **Quick-DNA™ MagBinding Buffer** to the sample and mix well.
7. Proceed to step 3 of DNA Purification on Page 7.

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<sup>1</sup> ZymoBIOMICS® DNA kits (D4300, D4302, D4303) are sold separately.

<sup>2</sup> Lysozyme (100 mg/mL) is available through Sigma-Aldrich (L2879-1G).

<sup>3</sup>  $\beta$ -Mercaptoethanol is available through Sigma-Aldrich (M6250-10ML)

<sup>4</sup> Alternatively, incubate at 37°C for 1 hour.

# Troubleshooting

Problem	Possible Causes and Solutions
<b>Low DNA Yield/Quality</b>	<b>Binding Time.</b> Make sure to incubate on rotator or shaker for 10 minutes after the <i>Quick-DNA™</i> MagBinding Buffer has been added to the sample. Incubation for longer periods of time may help to increase yield.
	<b>Proteinase K Digestion.</b> 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.
	<b>Resuspension of Beads.</b> The MagBinding Beads settle quickly. Ensure complete resuspension before use by thoroughly shaking and/or vortexing the bottle.
	Also, HMW DNA can sometimes cause the MagBinding Beads to clump, which can impact DNA binding and washing efficiency. If clumping is visible following the initial binding step (step 4), pipet mix until the clump is broken up and the beads fully resuspend.
	<b>Incomplete Microbial Lysis.</b> Some microbes may contain cell walls that are not susceptible to lysozyme or Zymolyase. Enzymatic digestion with other lytic enzymes may be required. Validation of additional enzymes is highly recommended to ensure complete microbial lysis is achieved.  The susceptibility of cell walls to lytic enzymes can be influenced by the age of the cultured cells. It is recommended to use fresh cells in early log phase, as they are more susceptible to lytic enzyme digestion. The optimal harvest time may require further optimization and validation.  <b>Amount of MagBinding Beads.</b> 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 of HMW DNA (sample type dependent).  <b>Prolonged Time or Increased Temperature.</b> 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 Concentration.** If the final concentration of your extracted DNA is too low, use 15  $\mu$ l of MagBinding Beads and 30  $\mu$ l of DNA Elution Buffer when processing similar samples in future.

### Low DNA Yield/Quality

**Incomplete Elution.** The recommended minimum elution volume is 1.5X ratio of the MagBinding Beads used (Ex. 50  $\mu$ l beads to 75  $\mu$ l 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  $\geq 55^{\circ}\text{C}$  during the minutes of shaking time may increase the 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
<b>Quick-DNA™ HMW MagBead Kit</b>	D6060	1 x 96 Preps.
Individual Kit Components	Catalog No.	Amount
<b>Proteinase K &amp; Storage Buffer</b>	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
	D3001-2-60	60 mg set
	D3001-2-125	125 mg set
<b>Biofluid &amp; Solid Tissue Buffer</b>	D4081-3-25	25 ml
	D4081-3-100	100 ml
<b>Quick-DNA™ MagBinding Buffer</b>	D4077-1-150	150 ml
	D4077-1-250	250 ml
<b>DNA Pre-Wash Buffer</b>	D3004-5-15	15 ml
	D3004-5-30	30 ml
	D3004-5-50	50 ml
	D3004-5-250	250 ml
<b>g-DNA Wash Buffer</b>	D3004-2-50	50 ml
	D3004-2-100	100 ml
	D3004-2-200	200 ml
	D3004-2-250	250 ml
	D3004-2-400	400 ml
<b>DNA Elution Buffer</b>	D3004-4-1	1 ml
	D3004-4-4	4 ml
	D3004-4-10	10 ml
	D3004-4-16	16 ml
	D3004-4-50	50 ml
<b>MagBinding Beads</b>	D4100-4-3	3 ml
	D4100-4-8	8 ml
	D4100-4-12	12 ml
	D4100-4-16	16 ml
	D4100-4-24	24 ml

# Complete Your Workflow

- ✓ To collect and transport samples at ambient temperatures:

DNA/RNA Shield™ and Collection Devices		
<a href="#">R1100</a>	DNA/RNA Shield™ Reagent	50 ml, 250 ml
<a href="#">R1200</a>	DNA/RNA Shield™ Reagent (2x Concentrate)	25 ml, 125 ml
<a href="#">R1150</a>	DNA/RNA Shield™ Blood Collection Tube	50 pack
<a href="#">R1160</a>	DNA/RNA Shield™ SafeCollect Swab Collection Kit	1 ml, 2 ml
<a href="#">R1211</a>	DNA/RNA Shield™ SafeCollect Saliva Collection Kit	2 ml

- ✓ Highly efficient and stable lytic enzymes for the digestion of yeast and fungal cell walls:

Zymolyase™		
<a href="#">E1005</a>	Zymolyase	2000 U
<a href="#">E1007-2</a>	Zymolyase Ultra	2000 U

- ✓ The *Quick-DNA*™ HMW MagBead (D6060) is compatible with automated platforms. For reliable automation solutions including consultations, scripts, evaluations, and implementations:

Contact an automation scientist at [automation@zymoresearch.com](mailto:automation@zymoresearch.com)

Learn more at [www.zymoresearch.com/pages/automation](http://www.zymoresearch.com/pages/automation).









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Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not 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.

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