ZYMO RESEARCH

DNA Purification Made Simple™

Quick-DNA™ Plant/Seed Miniprep Kit

Rapid isolation of ultra-pure DNA from plant tissue samples.

Highlights

- Simple method for the isolation of inhibitor-free, PCR-quality DNA (up to 25 µg/prep) from a variety of plant and seed samples in as little as 20 minutes.
- The eluted DNA is ideal for downstream molecular-based applications including PCR, arrays, endonuclease digestion, etc.
- State-of-the-art, ultra-high density ***BashingBeads***™ are fracture resistant and chemically inert.

Catalog Numbers:
D6020

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

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Product Contents

Quick-DNA™ Plant/Seed Miniprep Kit	D6020 (50 preps.)	Storage Temperature
ZR BashingBead™ Lysis Tubes (2.0 mm)	50	Room Temp.
BashingBead™ Buffer	40 ml	Room Temp.
Genomic Lysis Buffer[1]	100 ml	Room Temp.
DNA Pre-Wash Buffer[2]	15 ml	Room Temp.
g-DNA Wash Buffer	50 ml	Room Temp.
DNA Elution Buffer[3]	10 ml	Room Temp.
Zymo-Spin™ III-F Filters	50	Room Temp.
Zymo-Spin™ IV-IR HRC Filters[4]	50	Room Temp.
Zymo-Spin™ IICR Columns	50	Room Temp.
Collection Tubes	200	Room Temp.
Instruction Manual	1	-

[1] For optimal performance, add ~100% beta-mercaptoethanol to 0.5% (v/v) i.e., 500 µl per 100 ml.
[2] A precipitate may have formed in the **DNA Pre-Wash Buffe**r during shipping. To completely resuspend the buffer, incubate the bottle at 30-37 ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.
[3] The DNA Elution Buffer contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.
[4] HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter

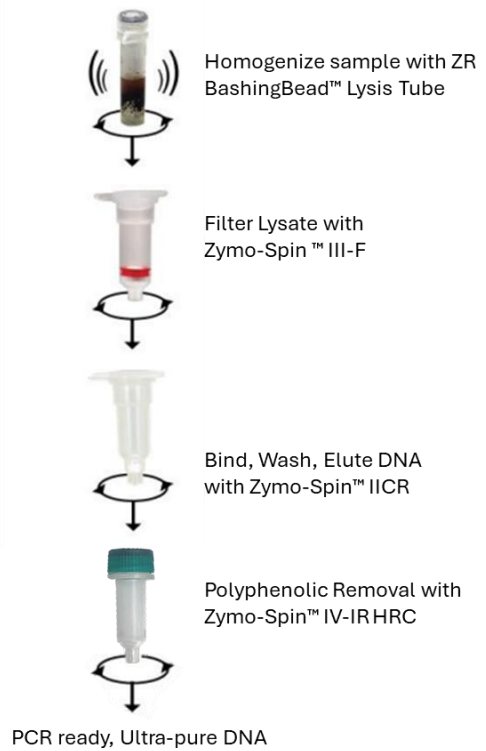
Specifications

- **Format** – Bead Beating, Spin Column.
- **Sample Sources** – Up to 150 mg that include leaves, stems, buds, flowers, fruit, seeds[1], etc.
- **DNA Yield** – Typically 20-80 ng of DNA/mg plant material.
- **DNA Purity** – High quality, inhibitor-free DNA is eluted with **DNA Elution Buffer** and is suitable for PCR amplification ($A_{260}/A_{280} > 1.8$).[2]
- **DNA Size Limits** – Capable of recovering genomic DNA up to and above 40 kb depending on bead beating parameters and initial sample quality. In most instances, mitochondrial DNA and viral DNA (if present) will also be recovered.
- **DNA Recovery** – Typically, up to 25 µg total DNA is eluted into 100 µl (50 µl minimum) **DNA Elution Buffer** per sample.
- **Equipment** – Microcentrifuge, vortex, cell disrupter/pulverizer (recommended).

[1] Seeds and vines are recommended to be pre-ground with mortar and pestle. If not possible to grind, seeds can be soaked overnight in water before bead beating.
[2] For microbiome or metagenomic applications, we recommend using the **ZymoBIOMICS® DNA** product line.

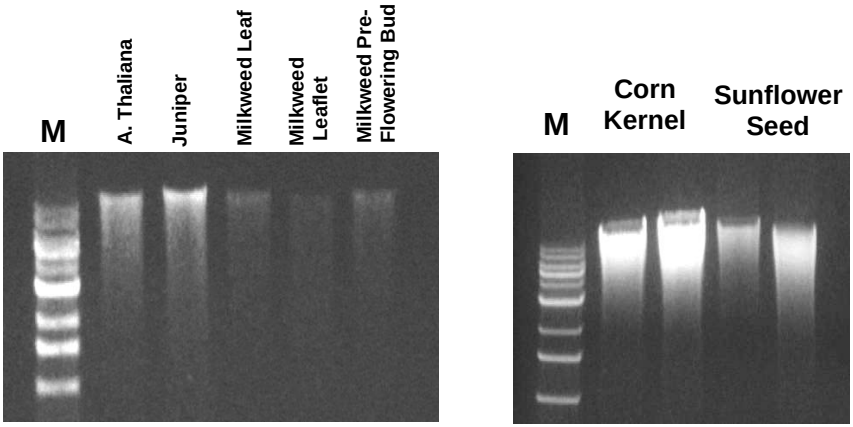
Product Description

The ***Quick*-DNA™ Plant/Seed Miniprep Kit**[1] is designed for the simple, rapid isolation of inhibitor-free, PCR-quality DNA from a variety of plant sample sources including leaves, stems, buds, flowers, fruit, seeds, etc. The procedure is easy and can be completed in as little as 20 minutes: plant samples (≤150 mg each) are added directly to a **ZR BashingBead™ Lysis Tube (2.0 mm)**[2] and rapidly and efficiently lysed by bead beating without the use of organic denaturants or proteinases. Polysaccharides and polyphenols/tannins are removed from the DNA using our Zymo-Spin™ Technology, which includes the **Zymo-Spin™ IV-IR HRC Filters**, a PCR inhibitor removal column. The eluted DNA is ideal for downstream molecular-based applications including PCR, arrays, etc. A schematic of the ***Quick*-DNA™ Plant/Seed Miniprep Kit** procedure is shown below.



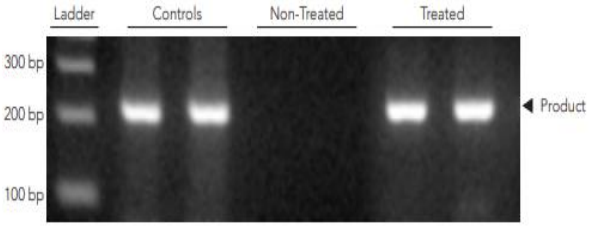
[1] For microbiome or metagenomic applications, we recommend using the **ZymoBIOMICS® DNA** product line.
[2] **DNA/RNA Shield™ (R1100-50, R1100-250)** can be used to stabilize nucleic acids and inactivate infectious agents in a variety of samples, without the need for reagent removal prior to extraction.

Superior Yields from Any Part of the Plant

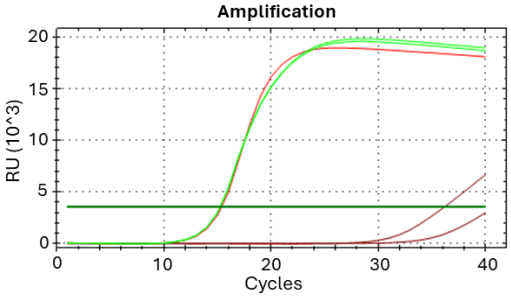


Comparison of DNA yields from various plant and seed samples using the ***Quick*-DNA™ Plant/Seed Miniprep Kit.** Equivalent amounts of plant materials were processed with equal volumes of eluted DNA analyzed in a 0.8% (w/v) agarose/ethidium bromide gel. **M** is a 1 kb DNA size marker (M5006-50).

Removal of Common Polyphenolics Found in Environmental Samples



Amplification of a 200bp product form DNA containing Humic Acid is restored after treatment with Zymo-Spin IV-IR HRC Column. PCR amplification was completely inhibited in the case of the "non-treated" sample. In each case equal amounts of DNA were used for each PCR and equivalent amounts of the reactions were then analyzed in a 2.0% (w/v) agarose/TAE/EtBr gel. The ladder is a 100 bp DNA marker (M5002-50). Hot start PCR was performed using ZymoTaq PreMix (E2003).



qPCR analysis of humic acid rich samples before and after treatment with Zymo-Spin IV-IR HRC Columns. Humic Acid Rich Samples were either untreated (brown) or treated (green) with Zymo-Spin IV-IR HRC columns. Samples were then analyzed with Femto Human qPCR Premix (E2005) and compared to DNA in water as positive control (red).

Protocol

For optimal performance, add ~100% 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.

1. Add up to 150 mg of finely cut plant or seed sample[1] to a **ZR BashingBead™ Lysis Tube (2.0 mm)**. Add 750 µl **BashingBead™ Buffer** to the tube and cap tightly.

   *For samples stored in **DNA/RNA Shield™**, see **Samples in DNA/RNA Shield™** (**pg. 6**).*

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.[2]

   ***Recommended:** Bead beat at max speed for 40 minutes using the Vortex Genie® 2 (S5001) with the Horizontal Microtube Holder (S5001-7). See **Optimized Lysis Protocols for Bead-Beating** (**pg. 7**) for additional lysis protocols.*

   ***Optional Stopping Point:** Samples can be stored after Step 2 at -80°C.*

3. Centrifuge the ZR BashingBead™ Lysis Tube (2.0 mm) in a microcentrifuge at 10,000 x *g* for 2 minutes.

4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ III-F Filter** in a **Collection Tube** and centrifuge at 8,000 x *g* for 1 minute. Discard the Zymo-Spin™ III-F Filter.

5. Add 1,200 µl of **Genomic Lysis Buffer** to the filtrate in the Collection Tube from Step 4. Mix well.

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IICR Column**[3] in a Collection Tube and centrifuge at 10,000 x *g* for 1 minute.

7. Discard the flow through from the Collection Tube and repeat Step 6.

8. Add 200 µl **DNA Pre-Wash Buffer** to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuge at ≥ 10,000 x *g* for 1 minute.

9. Add 500 µl **g-DNA Wash Buffer** to the Zymo-Spin™ IICR Column and centrifuge at ≥ 10,000 x *g* for 1 minute.

10. Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) **DNA Elution Buffer** directly to the column matrix. Centrifuge at ≥ 10,000 x *g* for 30 seconds to elute the DNA.

11. Loosen the screw cap from the **Zymo-Spin™ IV-IR HRC filter**[4] and break bottom tip off. Place in a clean Collection Tube and centrifuge at 8,000 x *g* for 3 minutes.

12. Transfer the eluted DNA to a prepared the Zymo-Spin™ IV-IR HRC filter in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 8,000 x *g* for 2 minutes.

    The filtered DNA is now suitable for PCR and other downstream applications.

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[1] Up to 1 ml of sample in **DNA/RNA Shield™** can be processed directly in **ZR BashingBead™ Lysis Tube**. Adjust final volume to 1 ml with **BashingBead™ Buffer** or **DNA/RNA Shield™**, if necessary.
[2] Processing time will vary based on sample input type and bead beater. Additional optimization may be required.
[3] The **Zymo-Spin™ IICR Column** has a maximum capacity of 800 µl.
[4] HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter.

Appendix

Samples in DNA/RNA Shield™

DNA/RNA Shield™ *ensures nucleic acid stability during sample storage and 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).*

1. For samples collected in DNA/RNA Shield™, transfer up to 1 ml of sample into the **ZR BashingBead™ Lysis Tube (2.0 mm)**.

   Note: If using < 1 ml sample, increase the volume to 1 ml using **BashingBead™ Buffer** or **DNA/RNA Shield™** before continuing.

2. Continue from Step 2 of the main protocol (**pg. 5**).

Optimized Lysis Protocols for Bead-Beating

The following conditions with different mechanical lysis machines were validated with minimum bias using the **ZymoBIOMICS® Microbial Community Standard**.

1 Vortex Genie with 2ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

2 Bertin Precellys Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed.

1. 1 minute on at 9,000 RPM
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

3 MP Fastprep-24™ (Classic & 5G) with 2 ml BashingBead™ Tubes

Maximum of 20 tubes. The weight of > 20 tubes may cause a system error.

1. 1 minute on at 6.5 m/s
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

4 Omni Bead Ruptor Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

1. 1 minute at maximum speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

6 Biospec Mini-BeadBeater-96 with 2 ml BashingBead™ Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

7 Biospec Mini-BeadBeater-96 with 96 well lysis rack

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

✕ TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

Troubleshooting

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

Problem	Possible Causes and Suggested Solutions
Background Contamination	**Contaminated workspace or equipment**. Clean workspace, centrifuge, and pipettes with 10% bleach routinely to avoid contamination. Make sure bags of columns and buffer bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
DNA Degradation	**DNase contamination:** Check pipettes, pipette tips, microcentrifuge tubes, etc. for DNase contamination and exercise the appropriate precautions during the DNA purification procedure. If water is used to elute the DNA, ensure that DNase-free water is used.
Low DNA Yield	**Incomplete sample lysis:** Bead beating devices that oscillate in a single dimension (only vertically or only horizontally) have been observed to inefficiently lyse very recalcitrant species. Devices that oscillate three-dimensionally or in a figure-8 motion often lyse efficiently. See **Optimized Lysis Protocols for Bead-Beating** (**pg. 7**) for recommended protocols. Dried samples may be hydrated in nuclease free water prior to processing to improve DNA extraction efficiency. **Incomplete debris removal:** For high density samples, ensure lysate is centrifuged properly to pellet insoluble debris following bead beating. Centrifugation duration can be increased to create a tighter pellet. Ensure that none of the debris is transferred to the **Zymo-Spin™ III-F Filter** in the next step. **Too much input material used.** If the lysate does not pass through the column or is extremely viscous, use less input material. Too much sample input can cause cellular debris to overload the column and insufficient flow. Consult the Sample Sources under Specifications for information on your input limit based on sample.

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	**Incomplete elution:** 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 60ºC before use. You can also load the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again.
Low DNA Purity	**Improper handling:** The column tip can be contaminated with wash buffer flow through. Ensure the tip does not touch the flow through. Empty the collection tube or use a new collection tube when instructed. **Insufficient centrifugation:** Ensure the indicated centrifugation times and speeds are used. Increase the centrifugation time of the final wash step by one minute to ensure complete wash buffer removal. Increase the centrifugation speed during the wash steps to 16,000 *x g*. **IV-IR HRC filter:** HRC filter chemistry will skew the A260/A230 purity ratio value. This will not affect any downstream processes. For accurate purity ratios, quantify sample prior to passing through the HRC filter.

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-DNA™ Plant/Seed Miniprep Kit**	D6020	50 preps.
***Quick*-DNA™ Plant/Seed 96 Kit**	D6021	2 x 96 preps.

Individual Kit Components	Catalog No.	Amount
ZR BashingBead™ Lysis Tubes	S6003-50	50 Pack
BashingBead™ Buffer	D6001-3-40 D6001-3-150	40 ml 150 ml
Genomic Lysis Buffer	D3004-1-50 D3004-1-100 D3004-1-150 D3004-1-200 D3004-1-250	50 ml 100 ml 150 ml 200 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 D3004-2-400	50 ml 100 ml 200 ml 250 ml 400 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
Zymo-Spin™ III-F Filters	C1057-50	50 Pack
Zymo-Spin™ IICR Columns	C1078-50	50 Pack
Zymo-Spin™ IV-IR HRC Filters	C1010-50	50 Pack
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 Pack 500 Pack 1000 Pack

Complete Your Workflow

✓ **Storage and Preservation of Nucleic Acids at Ambient Temperature**

DNA/RNA Shield™ and Collection Devices	Size	Catalog No.
DNA/RNA Shield™ Reagent	50 ml 250 ml	R1100-50 R1100-250
DNA/RNA Shield™ Reagent (2x Concentrate)	25 ml 125 ml	R1200-25 R1200-125
DNA/RNA Shield™ Lysis Tubes (Tissue)	50 pack	R1105

✓ **Reliable and Robust Amplification of DNA**

ZymoTaq DNA 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

✓ **Innovative Solution for Whole Genome Sequencing**

NGS Library Prep Kits	Size	Catalog No.
Zymo-Seq™ SPLAT DNA Library Kit	12 Preps.	D5464

✓ **All-Inclusive Genomic Sequencing Solutions**

Genomic Sequencing Services
Comprehensive genomic sequencing solutions from sample prep to analysis. Powered by PacBio HiFi and Illumina for accurate, high-resolution sequencing from even the most challenging samples. Contact services@zymoresearch.com for more information. Learn more at www.zymoresearch.com/pages/genomic-sequencing-services.

Notes

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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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