

Product Information

Extract-N-Amp™ Seed PCR Kit

Catalog Number **XNAS2**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

Technical Bulletin

Product Description

The Extract-N-Amp Seed PCR Kit contains all the reagents needed to rapidly extract and amplify genomic DNA from seeds (soybean, corn, wheat, etc.). Briefly, DNA is extracted from ground seed material by incubation in a mixture of Extraction Solution and Seed Preparation Solution at $55\text{ }^{\circ}\text{C}$ for 10 minutes. There is no need for organic extraction, column purification, or precipitation of the DNA. After the extraction is stopped by incubation at $95\text{ }^{\circ}\text{C}$ for 3 minutes, an equal volume of Neutralization Solution B is added and the extract is ready for PCR.

An aliquot of the neutralized extract is then combined with the Extract-N-Amp PCR ReadyMix™ and user-provided PCR primers to amplify target DNA. The Extract-N-Amp PCR ReadyMix is a 2× solution containing buffer, salts, dNTPs, and *Taq* DNA polymerase. It is optimized specifically for use with the extraction reagents. This formulation contains the JumpStart™ antibody for specific hot start amplification, but does not contain the inert red dye found in the REExtract-N-Amp™ PCR Reaction Mix to allow detection of PCR products by methods that are sensitive to the red dye.

Reagents provided

Sufficient reagents for 100 preparations

- Extract-N-Amp PCR ReadyMix, Catalog Number E3004, 1.2 ml. A 2× PCR reaction mix containing buffer, salts, dNTPs, *Taq* DNA polymerase and JumpStart antibody.
- Extraction Solution, Catalog Number E7526, 6 ml
- Neutralization Solution B, Catalog Number N3910, 6 ml
- Seed Preparation Solution, Catalog Number S1193, 0.9 ml

Reagents and equipment required, not provided

Items common to all procedures:

- Tubes or plates for PCR
- PCR primers
- Thermal Cycler
- Water, PCR Reagent, Catalog Number W1754

For individual 1.5 ml tubes:

- 1.5 ml microcentrifuge tubes
- Heat block or thermal cycler
- Pellet pestle, disposable, Catalog Number Z359947

For individual 1.5 ml tubes with liquid nitrogen

- 1.5 ml microcentrifuge tubes
- Liquid nitrogen
- Mortar and pestle
- Heat block or thermal cycler

For 96 well plates:

- Bead mill, 2000 Geno/Grinder from Spex Certiprep or equivalent
- 4 mm stainless steel grinding balls, Spex Certiprep
- 2 ml Square well block, Whatman Product Code 7701-5200
- 96 well sealing mat, Brinkmann Instruments Product Code 951-03-014-7
- 96 well PCR plate
- Thermal cycler
- Optional: Heat block with 96 well block

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

All components of the Extract-N-Amp Seed PCR Kit can be stored at 2-8 °C for up to 3 weeks. For long-term storage, -20 °C is recommended. Do not store in a "frost-free" freezer.

Procedures

All steps are carried out at room temperature unless otherwise noted.

A. Grinding Seeds

Following are three different methods for grinding seeds.

1. Grind using a Bead Mill

- 1a. Place 1 seed into each well of a 2 ml square well block.

Note: With *Arabidopsis* or similar sized seeds, approximately 50 seeds should be placed in a single well. This grinding procedure is not recommended for corn seeds, because results with such large, tough seeds are inconsistent

- 1b. Pipette PCR grade water into the well according to the following volumes:

800 µL for soybean or similar sized seeds
 600 µL for cotton or similar sized seeds
 200 µL for canola, sorghum, wheat, or similar sized seeds
 100 µL for *Arabidopsis* or similar sized seeds

- 1c. Place a 4 mm stainless steel grinding ball in each well of the 2 ml 96 square well block and cover with sealing mat. Place block in the bead mill and shake at 1,500 rpm for 10 minutes. Continue to **Section B**.

2. Grind individually using a Plastic Pestle

- 2a. Place 1 seed into a 1.5 ml microcentrifuge tube.

Note: With *Arabidopsis* or similar sized seeds, approximately 50 seeds should be placed in a single tube.

- 2b. Pipette PCR grade water into the well according to the following volumes:

800 µL for soybean or similar sized seeds
 600 µL for corn or similar sized seeds
 400 µL for cotton or similar sized seeds
 100 µL for *Arabidopsis*, canola, sorghum, wheat, or similar sized seeds

- 2c. Incubate the seed with water for 1 hour at 55°C.

- 2d. Grind hydrated seeds in tube using a plastic pestle. Continue to **Section B**.

3. Grind individually using liquid nitrogen

- 3a. Grind seed into a fine powder in liquid nitrogen using a mortar and pestle.

Note: With small seeds, such as *Arabidopsis* and canola, more than one seed must be ground to collect enough ground seed material.

- 3b. Transfer between 5 and 100 mg of ground seed material into a pre-weighed 1.5 ml microcentrifuge tube. Record the mass of the transferred seed material.

- 3c. Pipette 4 µL of water for every mg of transferred ground seed material into the sample tube and vortex to mix. Continue to **Section B**.

B. Extraction of Seeds

- Pipette 45 μL of Extraction Solution into a 1.5 ml microcentrifuge tube or multiwell PCR plate. Add 5 μL of Seed Preparation Solution to the tube and pipette up and down to mix.
Note: If several extractions will be performed, sufficient volumes of Extraction and Seed Preparation Solutions may be pre-mixed in a ratio of 9:1 up to 2 hours before use. The mixture should then be dispensed in 50 μL volumes into tubes or multiwell plates.
- Pipette 5 μL of the ground seed suspension from Section A into the Extraction Solution and Seed Preparation Solution mixture and vortex or pipette up and down to mix.
- Incubate the mixture at 55 °C for 10 minutes to extract DNA. Note that the ground seed will not appear to be digested at the end of this incubation; however, sufficient DNA will be released for PCR.
- Incubate the mixture at 95 °C for 3 minutes to stop the extraction.
Note: Steps 3 and 4 can be performed in a thermal cycler using a 96 well PCR plate.
- Add 50 μL of Neutralization Solution B to the mixture and vortex or pipette up and down to mix.
- Store the neutralized seed extract at 2-8 °C or continue to **Section C**.

C. PCR amplification

The Extract-N-Amp PCR ReadyMix contains JumpStart antibody for specific hot start amplification. Therefore, PCR reactions can be assembled at room temperature without premature *Taq* DNA polymerase activity.

Typical final primer concentrations are ~0.4 μM each. The optimal primer concentration and cycling parameters will depend on the system being used.

- Add the following reagents to a thin-walled PCR microcentrifuge tube or plate:

Reagent	Volume
Water, PCR Reagent	x μL
Extract-N-Amp PCR ReadyMix	10 μL
Forward primer	y μL
Reverse primer	y μL
Seed extract	4 μL^*
Total volume	20 μL

***Note:** The Extract-N-Amp PCR ReadyMix is formulated to compensate for components in the Extraction, Seed Preparation, and Neutralization B Solutions. If less than 4 μL of seed extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction and Neutralization B solutions to bring the volume of seed extract up to 4 μL .

- Mix gently and briefly centrifuge to collect all the components to the bottom of the tube.
- For thermal cyclers without a heated lid, add 20 μL of mineral oil to the top of each tube to prevent evaporation.
- The amplification parameters should be optimized for individual primers, template, and thermal cycler.

Common cycling parameters:

Step	Temperature	Time	Cycles
Initial Denaturation	94 °C	3 min	1
Denaturation	94 °C	0.5-1 min	30-35
Annealing	45 to 68 °C	0.5-1 min	
Extension	72 °C	1-2 min (~ 1 kb/min)	
Final Extension	72 °C	10 min	1
Hold	4 °C	Indefinitely	

- The amplified DNA can be loaded onto an agarose gel after the PCR is completed with the addition of a separate loading buffer/tracking dye such as Gel Loading Buffer, Catalog Number G2526.

Note: PCR products can be purified, if desired, for downstream applications such as sequencing with the GenElute™ PCR Clean-Up Kit, Catalog Number NA1020.

Troubleshooting Guide

Problem	Cause	Solution
Little or no PCR product is detected.	Seeds were not ground sufficiently.	For 96 well grind - Increase grinding time in bead mill. For seeds with a tough seed coat, it is helpful to break the seed before putting it into the plate to grind. For individual 1.5 ml tube - For seeds with a tough seed coat, it is helpful to break the seed before incubating it at 55 °C.
	PCR reaction may be inhibited due to contaminants in the seed extract.	Dilute the extract with a 50:50 mixture of Extraction and Neutralization B solutions. To test for inhibition, include a DNA control and/or add a known amount of template (100-500 copies) into the PCR along with the seed extract.
	A PCR component may be missing or degraded.	Run a positive control to insure that components are functioning. A checklist is also recommended when assembling reactions.
	There may be too few cycles performed.	Increase the number of cycles (5-10 additional cycles at a time).
	The annealing temperature may be too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers may not be designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 22 nucleotides long, try to lengthen the primer to 25-30 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%.
	The denaturation temperature may be too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time may be too long or too short.	Optimize the denaturation time by increasing or decreasing it in 10 second increments.
	The extension time may be too short.	Increase the extension time in 1 minute increments, especially for long templates.
	Target template is difficult.	In most cases, inherently difficult targets are due to unusually high GC content and/or secondary structure. Betaine solution, 5 M, Catalog Number B0300, has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.
Multiple products	JumpStart antibody is not working correctly.	Do not use DMSO or formamide with Extract-N-Amp PCR ReadyMix. It can interfere with the enzyme-antibody complex. Other cosolvents, solutes (e.g., salts), extremes in pH, or other reaction conditions may reduce the affinity of the JumpStart antibody for <i>Taq</i> polymerase and thereby compromise its effectiveness.
	“Touchdown” PCR may be needed.	“Touchdown” PCR ² significantly improves the specificity of many PCR reactions in various applications. Touchdown PCR involves using an annealing/extension temperature that is higher than the T_m of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer T_m for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.
Negative control shows a PCR product or “false positive” results are obtained.	Reagents are contaminated.	It is recommended that a reagent blank without DNA template be included as a control in every PCR run to determine if the reagents used in extraction or PCR are contaminated with a template from a previous reaction.

References

1. Dieffenbach, C.W., and Dveksler, G.S. (Eds.), PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1995). Catalog Number Z364118
2. Don, R.H. et al., 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.*, **19**, 4008 (1991).
3. Griffin, H.G., and Griffin, A.M. (Eds.), PCR Technology: Current Innovations, CRC Press, Boca Raton, FL (1994). Catalog Number Z357499
4. Innis, M.A., et al., (Eds.), PCR Strategies, Academic Press, New York (1995). Catalog Number Z364452
5. Innis, M., et al., (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, California (1990). Catalog Number P8177
6. Newton, C.R. (Ed.), PCR: Essential Data Series, John Wiley & Sons, New York (1995).
7. Roux, K.H. Optimization and troubleshooting in PCR. *PCR Methods Appl.*, **4**, 5185-5194 (1995).
8. Saiki, R., PCR Technology: Principles and Applications for DNA Amplification, Stockton, New York (1989).

Related Products

- Ethanol, Catalog Nos. E7148, E7023, and 459836
- Precast Agarose Gels, Catalog Nos. P5722, P6097, P6222, P5972, P5472
- PCR Marker, Catalog No. P9577
- TBE Buffer, Catalog Nos. T4415, T6400, and T9525
- Tubes for PCR, Catalog Nos. Z374873, Z374962 and Z374881

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

JumpStart and JumpStart Taq Antibody are licensed under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries

Extract-N-Amp, GenElute, JumpStart, ReadyMix, and REExtract-N-Amp are trademarks of Sigma-Aldrich™ Biotechnology LP and Sigma-Aldrich Co.

PHC 10/10-1