Product Information

Extract-N-Amp™ Seed PCR Kit

XNAS2

Storage Temperature -20 °C

Product Description

The Extract-N-Amp™ Seed PCR Kit for direct PCR contains 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 °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 °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 2x 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 REDExtract-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, Cat. No. E3004, 1.2 mL. A 2x PCR reaction mix containing buffer, salts, dNTPs, *Taq* DNA polymerase and JumpStart™ antibody.
- Extraction Solution, Cat. No. E7526, 6 mL
- Neutralization Solution B, Cat. No. N3910, 6 mL
- Seed Preparation Solution, Cat. No. S1193, 0.9 mL

Reagents and Equipment Required

(Not provided)

Items common to all procedures:

- Tubes or plates for PCR
- PCR primers, Cat. No. OLIGO
- Thermal Cycler
- Water, PCR Reagent, Cat. No. W1754

For individual 1.5 mL tubes:

- 1.5 mL microcentrifuge tubes
- Heat block or thermal cycler
- Pellet pestle, disposable, Cat. No. Z359955

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
- 4 mm stainless steel grinding balls
- 2 mL square well block
- 96-well sealing mat
- 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 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.

Grinding Seeds

Following are three different methods for grinding seeds.

Grind using a Bead Mill

1. 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 large, tough seeds are inconsistent.
2. 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
3. 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 Extraction of Seeds.

Grind individually using a plastic pestle

1. 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.
2. 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
3. Incubate the seed with water for 1 hour at 55 °C.
4. Grind hydrated seeds in tube using a plastic pestle. Continue to Extraction of Seeds.

Grind individually using liquid nitrogen

1. 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.
2. 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.
3. Pipette 4 µL of water for every mg of transferred ground seed material into the sample tube and vortex to mix. Continue to Extraction of Seeds.

Extraction of Seeds

1. Pipette 45 µL of Extraction Solution into a 1.5 mL microcentrifuge tube or multi-well 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 multi-well plates.
2. Pipette 5 µL of the ground seed suspension from Grinding Seeds into the Extraction Solution and Seed Preparation Solution mixture and vortex or pipette up and down to mix.
3. 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.
4. 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.
5. Add 50 µL of Neutralization Solution B to the mixture and vortex or pipette up and down to mix.
6. Store the neutralized seed extract at 2-8 °C or continue to PCR Amplification below.

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.

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

Reagent	Volume
Water, PCR Reagent	Variable
Extract-N-Amp™ PCR ReadyMix	10 µL
Forward primer	Variable
Reverse primer	Variable
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.

2. Mix gently and briefly centrifuge to collect all the components to the bottom of the tube.
3. For thermal cyclers without a heated lid, add 20 µL of mineral oil to the top of each tube to prevent evaporation.
4. 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 minutes	1
Denaturation	94 °C	30 seconds	
Annealing	45 to 68 °C	30 seconds	30-35
Extension	72 °C	1-2 minutes (1 min/kb)	
Final Extension	72 °C	10 min	1
Hold	4 °C	Indefinitely	

5. 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, Cat. No. G2526.

   Note: PCR products can be purified, if desired, for downstream applications such as sequencing with the GenElute™ PCR Clean-Up Kit, Cat. No. 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 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 ensure 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, Cat. No. B0300, has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.

Problem	Cause	Solution
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 *Taq* 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 Tm of the primers during the initial PCR cycles. The annealing/ extension temperature is then reduced to the primer Tm 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.	Include 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

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3. Griffin, H.G., and Griffin, A.M. (Eds.), PCR Technology: Current Innovations, CRC Press, Boca Raton, FL (1994).
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Tubes for PCR	Z374873, Z374962 and Z374881

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