

Product Information

Extract-N-Amp™ Plant PCR Kits

XNAP2, XNAP2E, XNAR

Product Description

The Extract-N-Amp™ Plant PCR Kits for direct PCR contain the reagents required to rapidly extract and amplify genomic DNA from plant leaves. Briefly, the DNA is extracted from a piece of leaf tissue, a 0.5 to 0.7 cm disk cut with a standard paper punch, by incubation in the Extraction Solution at 95 °C for 10 minutes. There is no need for freezing plant tissue in liquid nitrogen, mechanical disruption, organic extraction, column purification or precipitation of the DNA. After an equal volume of the Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR. An aliquot of the diluted extract is then combined with the Extract-N-Amp™ PCR Reaction Mix and user-provided PCR primers to amplify target DNA.

The Extract-N-Amp™ PCR ReadyMix is a 2X reaction mix containing buffer, salts, dNTPs, and *Taq* polymerase. It is optimized specifically for use with the extraction reagents. This formulation uses JumpStart™ *Taq* antibody for specific hot start amplification but does not contain the inert red dye found in the REExtract-N-Amp™ PCR ReadyMix to allow detection of PCR products by methods that are sensitive to the red dye.

Reagents Provided	Cat. No.	XNAP2	XNAP2E	XNAR
		100 extractions, 100 amplifications	100 extractions, 500 amplifications	1,000 extractions, 1,000 amplifications
Extraction Solution	E7526	12 mL	12 mL	120 mL
Dilution Solution	D5688	12 mL	12 mL	120 mL
Extract-N-Amp™ PCR ReadyMix, This is a 2X PCR reaction mix containing buffer, salts, dNTPs, <i>Taq</i> polymerase and JumpStart™ <i>Taq</i> antibody.	E3004	1.2 mL	5 x 1.2 mL	12 mL
Collection Tubes, 2 mL	T5449 or T7813	2 x 50 each	2 x 50 each	Not included

Reagents and Equipment Required

(Not Provided)

- Paper punch
- Forceps (small to medium in size)
- Heat block or water bath at 95 °C
- PCR Primers, Cat. No. OLIGO
- Water, PCR reagent, Cat. No. W1754

Precautions and Disclaimer

The Extract-N-Amp™ Plant PCR Kits are for R&D use only. Not for drug, household or other uses. Consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

The Extraction Solution, Dilution Solution and Extract-N-Amp™ PCR ReadyMix 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.

Procedure

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

DNA Extraction

1. Rinse the paper punch and forceps in 70% ethanol prior to use and between the handling of different samples.
2. Punch a 0.5 to 0.7 cm disk of leaf tissue into a 2 mL collection tube or suitable vessel using a standard one-hole paper punch. If frozen plant tissue is used, keep the leaves on ice while punching disks.
3. Add 100 µL of the Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the disk is covered by the Extraction Solution.
4. Incubate at 95 °C for 10 minutes. Note that leaf tissues usually do not appear to be degraded after this treatment.
5. Add 100 µL of the Dilution Solution and vortex to mix.
6. Store the diluted leaf disk at 2-8 °C. It is not necessary to remove the leaf disk before storage.

PCR Amplification

The Extract-N-Amp™ PCR ReadyMix contains JumpStart™ *Taq* antibody for specific hot start amplification. Therefore, PCR mixtures 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
Leaf disk extract	4 µL *
Total volume	20 µL

*The Extract-N-Amp™ PCR ReadyMix is formulated to compensate for components in the Extraction and Dilution Solutions. If less than 4 µL of leaf disk extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction and Dilution Solutions to bring the volume of leaf disk extract up to 4 µL.

2. Mix gently and briefly centrifuge to collect all the components at 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	
Extension	72 °C	1-2 minutes (1 min/kb)	30-35
Final Extension	72 °C	10 minutes	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

Problem	Cause	Solution
Little or no PCR product is detected	PCR reaction may be inhibited due to contaminants in the plant extract.	Dilute the extract with a 50:50 mix of extraction and dilution solutions. To test for inhibition, include a DNA control and/or spike a known amount of template (100-500 copies) into the PCR along with the plant 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 by 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 by 1 °C increments.
	The denaturation time may be too long or too short.	Optimize the denaturation time by increasing or decreasing it by 10 second increments.
	The extension time may be too short.	Increase the extension time by 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 (Cat. No. B0300) has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.
Multiple products	JumpStart™ <i>Taq</i> 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) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart™ <i>Taq</i> antibody for <i>Taq</i> polymerase and thereby compromise its effectiveness.
	Touchdown PCR may be needed.	"Touchdown" PCR 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.
Contamination	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.

Product Ordering

Order products online at [SigmaAldrich.com](https://www.sigmaaldrich.com).

Related Products	Cat. Nos.
Tubes for PCR	Z374873, Z374962 and Z374881
PCR Marker	P9577
Precast Agarose Gels	P6097
TBE Buffer	T4415, T6400 and T9525
Ethanol	E7148, E7023 and 459836

References

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