

Product Information

Extract-N-Amp™ Plant PCR Kits

Product Codes **XNAP2**, **XNAP2E**, and **XNAR**

TECHNICAL BULLETIN

Product Description

The Extract-N-Amp™ Plant PCR Kits contain all of 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	Product Code	XNAP2 100 extractions, 100 amplifications	XNAP2E 100 extractions, 500 amplifications	XNAR 1,000 extractions, 1,000 amplifications
Extraction Solution	E 7526	12 ml	12 ml	120 ml
Dilution Solution	D 5688	12 ml	12 ml	120 ml
Extract-N-Amp PCR ReadyMix, This is a 2x PCR reaction mix containing buffer, salts, dNTPs, Taq polymerase and JumpStart Taq antibody.	E 3004	1.2 ml	5 x 1.2 ml	12 ml
Collection Tubes, 2 ml	T 7813	2 x 50 each	2 x 50 each	Not included

Reagents and Equipment Required But Not Provided

- Paper punch
- Forceps (small to medium in size)
- Heat block or water bath at 95 °C
- PCR Primers
- Water, PCR reagent, Product Code W 1754

Precautions and Disclaimer

The Extract-N-Amp Plant PCR Kits are for laboratory use only. Not for drug, household or other uses. Consult the MSDS 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 on a short-term basis, but 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.

A. DNA extraction

1. Rinse the paper punch and forceps in 70% ethanol prior to use and between the handling of different samples.

- 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.
- Add 100 μ l of the Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the disk is covered by the Extraction Solution.
- Incubate at 95 °C for 10 minutes. Note that leaf tissues usually do not appear to be degraded after this treatment.
- Add 100 μ l of the Dilution Solution and vortex to mix.
- Store the diluted leaf disk at 2-8 °C. It is not necessary to remove the leaf disk before storage.

B. PCR amplification

The Extract-N-Amp PCR ReadyMix contains JumpStart Taq 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 μ M 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 μ l
Extract-N-Amp PCR reaction mix	10 μ l
Forward primer	y μ l
Reverse primer	y μ l
Leaf disk extract	4 μ l*
Total volume	20 μ l

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

- Mix gently and briefly centrifuge to collect all the components at the bottom of the tube.

- For thermal cyclers without a heated lid, add 20 μ l 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 minutes	1
Denaturation	94 °C	0.5-1 minutes	30-35
Annealing	45 to 68 °C	0.5-1 minutes	
Extension	72 °C	1-2 minutes (~ 1 kb/min)	
Final Extension	72 °C	10 minutes	1
Hold	4 °C	Indefinitely	

References

- Dieffenbach, C. W. and Dveksler, G. S. (Eds.) *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, New York, 1995). (Product Code Z36,411-8)
- Don, R. H. et al. 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.*, **19**, 4008 (1991)
- Erich, H. A. (Ed.) *PCR Technology: Principles and Applications for DNA Amplification* (Stockton Press, New York, 1989). (Product Code P 3551)
- Griffin, H. G. and Griffin, A. M. (Eds.) *PCR Technology: Current Innovations*, (CRC Press, Boca Raton, FL, 1994) (Product Code Z35,749-9)
- Innis, M.A., et al. (Eds.) *PCR Strategies* (Academic Press, New York, 1995) (Product Code Z36,445-2)
- Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, San Diego, California, 1990) (Product Code P 8177)
- McPherson, M.J. et al. (Eds.) *PCR 2: A Practical Approach* (IRL Press, New York, 1995) (Product Code Z36,238-7)
- Newton, C.R. (Ed.) *PCR: Essential Data*, (John Wiley & Sons, New York, 1995) (Product Code Z36,491-6)
- Roux, K.H. Optimization and troubleshooting in PCR. *PCR Methods Appl.*, **4**, 5185-5194 (1995).
- Saiki, R., *PCR Technology: Principles and Applications for DNA Amplification* (Stockton, New York, 1989)

Troubleshooting Guide

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 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 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 (Product Code B 0300) has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.
Multiple products	JumpStart Taq 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 Taq antibody for Taq 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.	Sigma recommends 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.

Related Products Product Code

Tubes for PCR Z37,487-3, Z37,496-2 and Z37,488-1

Thermowell PCR Plate P 3606

Reusable sealing mat for 96 well plate P 4356

PCR Marker P 9577

Precast Agarose Gels P 5597, P 5847, and P 6097

TBE Buffer T 4415, T 6400 and T 9525

Ethanol E 7148, E 7023 and 45,983-6

NOTICE TO PURCHASER: LIMITED LICENSE

A license under US Patents 4,683,202, 4,683,195, 4,965,188, and 5,075,216 or their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the Polymerase Chain Reaction ("PCR") and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. Rights to the up-front fee component must be obtained by the end user in order to have a complete license to use this product in the PCR process. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an Authorized Thermal cycler. No right to perform or offer commercial services of any kind using PCR, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or the Licensing Department at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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

JWM 5/29/03

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.