**SIGMA-ALDRICH®**

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Extract-N-Amp™ Plant PCR Kits**

Catalog Numbers **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 REDExtract-N-Amp™ PCR ReadyMix to allow detection of PCR products by methods that are sensitive to the red dye.

| Reagents Provided | Catalog Number | **XNAP2** 100 extractions, 100 amplifications | **XNAP2E** 100 extractions, 500 amplifications | **XNAR** 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, Taq polymerase and JumpStart Taq 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 But Not Provided

- Paper punch
- Forceps (small to medium in size)
- Heat block or water bath at 95 °C
- PCR Primers
- Water, PCR reagent, Catalog Number W1754

### 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.
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.

**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 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 | x μL |
| Extract-N-Amp PCR ReadyMix | 10 μL: |
| Forward primer | y μL |
| Reverse primer | y μL |
| Leaf disk extract | 4 μL* |
| Total volume | 20 μL |

*Note: 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: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 | 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**


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. Erlich, H. A. (Ed.) *PCR Technology: Principles and Applications for DNA Amplification* (Stockton Press, New York, 1989).
4. Griffin, H. G. and Griffin, A. M. (Eds.) *PCR Technology: Current Innovations*, (CRC Press, Boca Raton, FL, 1994) Catalog Number Z357499
5. Innis, M.A., et al. (Eds.) *PCR Strategies* (Academic Press, New York, 1995). Catalog Number Z364452
6. Innis, M., et al. (Eds.) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, San Diego, California, 1990). Catalog Number P8177
7. McPherson, M.J. et al. (Eds.) *PCR 2: A Practical Approach* (IRL Press, New York, 1995) Catalog Number Z362387
8. Newton, C.R. (Ed.) *PCR: Essential Data*, (John Wiley & Sons, New York, 1995).
9. Roux, K.H. Optimization and troubleshooting in PCR. *PCR Methods Appl.*, **4,** 5185-5194 (1995).
10. 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 | Catalog Numbers |
|---|---|
| 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 |

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

JC,PHC 06/11-1

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

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.