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

SYBR® Green Extract-N-Amp™ Plant PCR Kit

Catalog Numbers **XNAPSG, XNAPG and XNAPRG**

TECHNICAL BULLETIN

Product Description
The SYBR Green Extract-N-Amp Plant PCR Kit contains the reagents needed for rapid extraction, amplification and detection of genomic DNA from plant leaves. DNA is rapidly extracted from a piece of leaf tissue by incubation in 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 Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for real-time PCR in any plate-based real-time thermal cycling system.

An aliquot of the neutralized extract is then combined with the Extract-N-Amp SYBR Green PCR ReadyMix™ and user-provided PCR primers. The Extract-N-Amp SYBR Green PCR ReadyMix is a 2x reaction mix containing SYBR Green, buffer, salts, dNTPs, Taq DNA Polymerase, and JumpStart™ Taq antibody. It is optimized specifically for use with the extraction reagents and contains JumpStart Taq antibody for hot start PCR to enhance specificity and SYBR Green I to act as a nonspecific reporter for real-time PCR.

Reagents Provided	Catalog Number	XNAPSG 10 extractions; 10 amplifications	XNAPG 100 extractions; 100 amplifications	XNAPRG 1,000 extractions; 1,000 amplifications
Extraction Solution	E7526	1.2 ml	12 ml	120 ml
Dilution Solution	D5688	1.2 ml	12 ml	120 ml
SYBR Green Extract-N-Amp PCR ReadyMix. **This is a 2x real-time PCR reaction mix** containing SYBR Green, buffer, salts, dNTPs, Taq DNA Polymerase and JumpStart Taq antibody.	S4320	0.15 ml	1.2 ml	12 ml

Reagents and equipment required, not provided
- Paper punch
- Forceps (small to medium in size)
- Collection Tubes, 2 ml
- Heat block or water bath at 95 °C
- PCR primers
- Water, PCR reagent, Catalog Number W1754

Related Products	Catalog Numbers
Collection Tubes, 2 mL	T7813
Tubes for PCR	Z374873, Z374962 or Z374881
PCR Marker	P9577
Precast Agarose Gels	P6097
TBE Buffer	T4415, T6400 or T9525
Ethanol (95-100%)	E7148, E7023 or 45,983-6
Extract-N-Amp PCR Diluent	E8155

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/Stability
The Extraction and Dilution Solutions can be stored at 2-8 °C up to 3 weeks; if storing longer than 3 weeks keep the solutions at –20 °C. Do not store in a "frost-free" freezer.

The Extract-N-Amp SYBR Green PCR ReadyMix should always be kept at –20 °C and out of light as much as possible. Excessive freeze-thawing of the ReadyMix can be detrimental to product performance. Aliquot the ReadyMix into suitably sized portions, if necessary, to avoid more than five freeze-thaws.

Preliminary Considerations

Primer Design and Optimization

SYBR Green I will detect both specific and non-specific PCR amplicons. Well-designed primers are recommended to ensure the highest possible specificity. Primers for PCR should be designed with the aid of primer design software to eliminate the complications introduced with primer-dimers, non-specific hybridization and secondary structures. The size of the PCR target should preferably be less than 500 bp, although this product has performed with targets up to 1000 bp. Larger targets are often harder to quantify via real-time PCR.

The optimal primer concentration and cycling parameters need to be optimized and will depend on the system being used. Typical final primer concentrations are ~0.4 µM each. Lower primer concentrations may decrease the accumulation of primer-dimer formation and nonspecific product formation.

For further information on primer design and optimization, consult the manufacturer of your thermal cycling system for assistance.

Controls

A positive control is always helpful to verify that the PCR reaction is performing properly. Use purified genomic DNA from the same plant species and dilute to 1-5 ng/µl with a 50:50 mixture of Extraction and Dilution Solutions or Extract-N-Amp PCR Diluent, Catalog Number E8155. **Do not use water to dilute the positive control**. Replace the 4 µL of leaf extract with 4 µL of the positive control in a 20 µL PCR reaction (see step B1).

A negative control is necessary to determine if contamination or primer-dimer formation is present. Replace the 4 µL of leaf extract with 4 µL of a 50:50 mixture of Extraction and Dilution Solutions or Extract-N-Amp PCR Diluent in a 20 µL PCR reaction (see step B1). **Do not use water as a negative control**.

Procedure

All steps are carried out at room temperature unless otherwise noted. All reagents should be completely thawed, brought to room temperature and gently mixed before use.

A. DNA Extraction

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

2. Punch Leaf Disk
   Punch a 0.5 to 0.7 cm disk of leaf tissue into a 2 ml collection tube using a standard one-hole paper punch. If frozen plant tissue is used, keep the leaves on ice while punching disks.

Note: Alternatively, if a one-hole paper punch is not available, tear or cut a piece of leaf tissue equivalent to a 0.5 to 0.7 cm disk.

3. Extract
   Add 100 µL of Extraction Solution to the collection tube. Close the tube and vortex briefly. Make sure the Extraction Solution completely covers the leaf disk.

4. Incubate
   Incubate at 95 °C for 10 minutes. Leaf tissues may not appear degraded after this treatment.

Note: To ensure the best possible extraction use a heat block or water bath that fits the contour of the tube used for extraction. Heating in an oven does not provide efficient heat conduction. Increase incubation time if a heat block or water bath is not available. Any additional time needed must be determined empirically.

5. Neutralize
   Add 100 µL of Dilution Solution to the collection tube. Close the tube and vortex briefly.

6. Store
   Store the neutralized leaf extract at 2-8 °C. It is not necessary to remove the leaf disk before storage. Neutralized leaf extracts have demonstrated stability at 2-8 °C storage for at least two years.

B. Real-Time PCR Amplification

The Extract-N-Amp SYBR Green 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. See reference 1 for general information regarding real-time PCR.

With some plants, PCR may be inhibited by secondary metabolites in the leaf extract such that little or no detectable PCR product is obtained. Diluting the leaf extract five-, ten-, or twenty-fold with a 50:50 mixture of Extraction and Dilution Solutions or with Extract-N-Amp PCR Diluent has been shown to alleviate PCR inhibition in some cases.

Note: The reagents included in this kit were formulated together and are dependent on one another for optimal performance of this product. Therefore, only use the recommended diluents instead of water to dilute leaf extracts.

1. Prepare Reaction Mixture
   Add the following reagents to a thin-walled PCR microcentrifuge tube or plate appropriate for the instrument to be used. Be aware that many instruments require an internal reference dye, which should be included in the reaction mixture.

PCR Reaction Setup

Reagent	Volume/1 RXN
SYBR Green Extract-N-Amp PCR ReadyMix	10 µL
Water, PCR Reagent	*x* µL
Forward Primer	*y* µL
Reverse Primer	*y* µL
Leaf Extract	4 µL
Total Volume	20 µL

   Alternatively, the total volume of a PCR reaction can be adjusted by scaling the reagents relative to the desired PCR total volume.

   When preparing multiple PCR reactions, it may be beneficial to create a master-mix. Add an excess number of reactions to account for measuring losses. After determining the appropriate volume for all reagents, combine and gently vortex to create master-mix. Add 16 µl of master mix and 4 µl of leaf extract to each reaction.

2. Mix and Centrifuge
   Mix gently and briefly centrifuge to collect all components at the bottom of the tube or plate.

3. Amplification Parameters
   The amplification parameters should be optimized for individual primers, template, and thermal cyclers with real-time SYBR Green detection.

Common Cycling Parameters

Step	Temperature	Time	Cycles
Initial Denaturation	94 °C	3 minutes	1
Denaturation	94 °C	15 seconds – 1 minute	30-40
Annealing	45 to 68 °C	15 seconds – 1 minute	
Extension	72 °C	10 seconds – 1 minute (~1 min/kb)	

4. Data Analysis
   Follow the recommendations of the real-time instrument used to effectively analyze results. Run a melt curve to ensure the correct PCR target was amplified.[1] For further verification, run the PCR reaction on a 2% Agarose-TBE buffered gel.

References

1. Ririe K. M., et al., Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. *Anal. Biochem*., **245,** 154-60 (1997).

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: 5,994,056 and 6,171,785.. 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 (such as apparatus or system claims in US Patent No. 6,814,934) 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.

SYBR Green I is a registered trademark and its use protected under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries.

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

PHC 10/10-1

Troubleshooting Guide

Problem	Possible Cause	Solution
No PCR product (no fluorescence detected) in sample and positive control	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%.
	A PCR component may be missing or degraded	A checklist is recommended when assembling reactions. Ensure the DNA isn't degraded in the positive control. Run a 2% Agarose TBE-buffered gel to confirm if the target was amplified, but not detected.
	The annealing temperature may be too high.	Decrease the annealing temperature in 2-4 °C increments.
	The extension time may be too short.	Increase the extension time in 30 second increments, especially for longer templates.
	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 the time in 10 second increments.
	There may be too few cycles performed.	Increase the number of cycles (5-10 additional cycles at a time).
	Target template is difficult.	In most cases, inherently difficult targets are due to unusually high GC content and/or secondary structure. Betaine has been reported to help amplification of high GC content templates at a concentration of 1.0-1.7 M.
No PCR product (signal) in sample, but there is product in positive control	The DNA extraction was inadequate	Repeat the extraction; thaw all kit reagents, bring to room temperature and thoroughly mix all kit reagents prior to use. Increase length of incubation and/or temperature if normal extraction protocol is inadequate.
	PCR reaction may be inhibited due to secondary metabolites in the leaf extract.	Dilute the extract with a 50:50 mix of Extraction and Dilution Solutions or Extract-N-Amp PCR Diluent , Catalog Number E8155.
Multiple PCR products	JumpStart Taq antibody is not working correctly.	Do not use DMSO or formamide with Extract-N-Amp SYBR Green 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 uses 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. Note that fluorescence is inversely proportional to temperature, thus fluorescence must be read at the same temperature every cycle.
	Primer-dimers are co-amplified	Include an additional detection step in the cycling program to avoid detection of primer-dimers, or if possible increase the temperature where fluorescence is read.
	Primer concentration is too high.	Reduce the primer concentration in a series of two-fold dilutions (i.e. 0.2 μM, 0.1 μM, 0.05 μM) and test in a trial set of PCR reactions.
	Primers are degraded.	Check for primer degradation on a polyacrylamide gel.
Negative Control shows PCR product (signal)	Reagents or reactions have been contaminated	Extraneous DNA template may have been introduced into the reagents or when setting up the PCR reactions. Clean the area in which PCR is setup. Then, rerun the experiment being careful not to contaminate the reactions. If product still amplifies in negative controls, the reagents were probably contaminated and should be replaced with unused reagents.

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.