User Guide

SYBR Green Extract-N-Amp™ PCR Ready Mix

S4320

Product Description

The SYBR Green Extract-N-Amp™ PCR Ready Mix is the specially formulated PCR enzyme blend contained in the SYBR Green Extract-N-Amp™ Plant PCR kit and SYBR Green Extract-N-Amp™ Tissue PCR Kit. It is intended to supplement these kits for researchers who perform more than one amplification per extracted sample.

This is a 2X real-time PCR reaction mix containing SYBR Green, buffer, salts, dNTPs, *Taq* polymerase and JumpStart™ *Taq* antibody. The neutralizing monoclonal antibody binds to Taq DNA polymerase, inactivating it until the complex is dissociated at ≥ 70 °C, providing simple and efficient hot start PCR. Because Jumpstart™ *Taq* DNA polymerase is inactive at room temperature, reaction mixtures can be prepared on the bench and transported directly to the thermocycler as the enzyme will become active in the first denaturation step of the cycling process. The SYBR Green double stranded DNA (dsDNA) stain acts as a nonspecific reporter for real-time PCR.

Features

- Optimized for use with Extract-N-Amp™ lysis reagents
- Hotstart feature allows for room temperature set-up without premature *Taq* DNA polymerase activity
- Ready Mix allows for decreased pipetting which saves time, reduces risk of contamination, and improves reproducibility
- SYBR Green stain included for tracking dsDNA amplification

Applications

- PCR
- Real-time quantitative PCR (qPCR)

After extraction using Extract-N-Amp™ reagents SYBR Green Extract-N-Amp™ PCR Ready Mix can be used for amplification of:

- Genomic DNA
- cDNA
- Low copy number targets
- Multiple targets (multiplex PCR)

Materials and Reagents Required

(Not included, see Product Ordering)

- Sample containing template DNA
- Extract-N-Amp™ reagents
- Microcentrifuge tubes or multi-well plate for extractions (200 µL minimal volume)
- Nuclease-free water
- Custom ordered primers specific to gene target
- PCR tubes or plates
- Thermal cycler capable of real-time SYBR Green detection

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/Stability

Keep at –20 °C away from light. Aliquot the Ready Mix into suitably sized portions if necessary to maintain product performance.

Directions for Use

Assemble Reaction Mix

If amplifying the same target from different samples, assemble a master mix of PCR reagents by multiplying the number of reactions needed (plus 10% to account for pipetting error) by the suggested volumes in the table below. Aliquot reaction mixture into PCR tubes. PCR reactions can be assembled at room temperature.

Reagent	Final Concentration	Amount per 20 µL reaction
SYBR Green Extract-N-Amp™ PCR Ready Mix	1X	10 µL
Primers	0.1–0.5 µM	Variable
Neutralized extract	Variable	4 µL
Nuclease-free Water	-	To 20 µL total

Note: Optimal concentration of primers and amount of extract will be target-specific. For more information on ideal concentrations based on application see Technical Guide.

Add Sample

Add the 4 µL of neutralized sample extract and mix gently.

Amplify

A suggested thermocycling protocol is provided below:

Initial denaturation		94 °C	2 minutes
40 cycles	Denaturation	94 °C	15 seconds
	Annealing	60 °C or 5 °C below lowest primer T_m	30 seconds
	Extension	72 °C	1 minute/kb
Final extension		72 °C	1 minute
Hold		4 °C	∞

Amplification parameters will vary depending on primers, template, and instrument used. For tips on optimizing PCR conditions as well as a 2-step cycling protocol please see the Technical Guide section.

Evaluate

Amplified DNA can be evaluated by standard methods, such as agarose gel electrophoresis, qRT-PCR and DNA sequencing.

Note: 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.

Technical Guide

Considerations for Primer Design

Thoughtful primer design is essential for PCR efficiency and specificity. For successful amplification consider the following:[1]

- Select an 18-30 nucleotide-long sequence with 40-60% G/C content and even distribution of all 4 bases.
- Avoid repetitive elements or self-complimentary sequences > 3 bp.
- Primer pairs should not differ in length by > 3 bp and should not contain complementarity to one another.
- Maintain calculated primer T_m between 55-60 °C, permitting only 2-3 °C variation between primer pairs. Priming efficiency can be increased by including a terminal G at the 3' end; however, the number of Gs or Cs in the last 5 bases of the primer sequence should be no more than 3.
- Ensure each primer sequence is unique to the gene of interest and is absent in other genes in the gDNA sample or within the vector.

Optimization of PCR Conditions

PCR involves the cycling of denaturing, annealing, and extension steps for DNA synthesis by a polymerase enzyme. To obtain the best product yield and accuracy, each step must be optimized.

The denaturing step (94-96 °C) activates the JumpStart™ *Taq* DNA Polymerase and separates double-stranded DNA strands, making it accessible to primers.[1] The duration of this step should be long enough to denature DNA but not so long that it compromises *Taq* DNA polymerase integrity. High salt conditions, GC-rich (> 55%) templates, and gDNA templates may require longer denaturation times and/or higher temperatures. For maximum retention of JumpStart™ *Taq* activity during thermocycling, use 94 °C for denaturation.

The annealing temperature can be calculated by subtracting 5 °C from the lowest reaction primer T_m The annealing time should be long enough for the primer to anneal to the template but not too long for non-specific annealing to occur.[1]

The optimal extension temperature for *Taq* DNA Polymerase is 72 °C; however, lower temperatures may be used for some reactions. Extension time depends on the length and complexity of the target sequence. For complex templates, use 1 minute/kb, with 15 seconds added if the PCR product is > 2 kb. Short or non-complex templates may be amplified with extension times of 30 seconds/kb.

The number of cycles needed for amplification depends on the amount of template input, with higher amount of input requiring less cycling. Generally, 25-30 cycles are sufficient to produce detectable product; however, low concentration templates may require up to 45 cycles.

To maintain enzyme fidelity, or accuracy of nucleotide incorporation, limit the number of PCR cycles and use an equimolar concentration of each dNTP. A low magnesium concentration is also important to maintain enzyme fidelity.[2]

Handling gDNA Templates

To prevent genomic DNA (gDNA) shearing, add template last and mix gently using a wide pore pipet tip. DO NOT VORTEX!

Two-Step PCR Amplification

Application of a two-step PCR process is possible when the annealing and extension temperatures are similar.

Initial denaturation		94 °C	3 minutes
40 cycles	Denaturation	94 °C	3 seconds
	Annealing/ extension	60 °C*	15-30 seconds
Hold		4 °C	∞

*Consult primer T_m regarding temperature selection. Extension time is target dependent, with larger targets requiring more than the recommended time.

Controls

For a positive control, use purified genomic DNA from the same species as the extract and dilute to 1-5 ng/µL with a 50:50 mixture of Extraction and Neutralization Solutions or Extract-N-Amp™ PCR Diluent. Do not use water to dilute the positive control. Replace the 4 µL of extract with 4 µL of the positive control in a 20 µL PCR reaction.

A negative control is necessary to determine if contamination or primer-dimer formation is present. Replace the 4 µL of extract with 4 µL of appropriate sample dilution buffer (see table in Sample Dilution section of this technical guide) in a 20 µL PCR reaction. Do not use water as a negative control.

Include a DNA control and/or add a known amount of template (100-500 copies) into the PCR mixture along with sample extract to test for inhibition inhibited due to contaminants in the extract. If PCR is inhibited in this control sample, use less extract or dilute the extract as described in the Sample Dilution section of this technical guide.

Sample Dilution

PCR may be inhibited by secondary metabolites in the extract, which can be overcome by sample dilution or supplementation with neutralization solution. The SYBR Green Extract-N-Amp™ PCR Ready Mix was formulated together with Extract-N-Amp™ reagents, and both are therefore dependent on one another for optimal performance. Unless otherwise noted, the use of water for sample dilution is not recommended; thus, if sample dilution is necessary, use the following conditions:

Extract Source	Dilutant
Plant	50:50 mixture of Extraction and Dilution Solutions or with Extract-N-Amp™ PCR Diluent
Tissue/Hair/ Saliva/Buccal swab	50:50 mixture of Extraction and Neutralization B Solutions or with Extract-N-Amp™ PCR Diluent

Recommended $MgCl_2$ Concentration

Because JumpStart™ *Taq* DNA Polymerase is a magnesium ion-dependent enzyme, the optimal concentrations of template DNA, primers, and $MgCl_2$ will be target-dependent. The optimal $MgCl_2$ concentration is also dependent upon the intended application. Reactions can be supplemented with up to 0.5 mM of additional $MgCl_2$ for an endpoint experiment, and up to 2 mM for a qPCR experiment.

PCR-Enhancing Additives

When optimizing PCR conditions for a new experiment, the following can be added to the reaction mix individually. After performing PCR amplification, samples with and without additive can be compared using agarose gel electrophoresis or other standard methods to look for improved product specificity and yield.

Additive	Purpose
BSA (10-100 µg/mL)	*Taq* DNA polymerase stabilization[2]
Formamide (1.25-10%)	Increases specificity in G/C rich regions[3]
DMSO (Up to 5%)	Accelerates strand renaturation[4] Nucleic acid thermal stability against depurination[4]
Glycerol (Up to 10%)	Increases thermal stability of the polymerase and lowers the temperature necessary for strand separation[4]
Ammonium sulfate (15-30 mM)	Affects the denaturing and annealing temperatures of the DNA[5]
Single strand binding protein (0.7-1.5 µg)	Inhibits formation of secondary structures, improving fidelity and *Taq* processivity[6]
Betaine (0.8-1.6 M)	Reduces base pair composition dependence of DNA melting[7]

Troubleshooting Guide

Problem	Suggestions
No or low product amplification	Titrate $MgCl_2$ concentration in 0.5 mM increments molecular biology grade $MgCl_2$ (see Product Ordering). See Recommended $MgCl_2$ Concentration section in the Technical Guide for expected concentration ranges based on application. Each amplicon target must be optimized individually.
	PCR may be inhibited by secondary metabolites in the extract. Diluting the extract five-, ten- or twenty-fold with a 50:50 mixture of Extraction and Neutralization Solutions or with Extract-N-Amp™ PCR Diluent has been shown to alleviate PCR inhibition. See Technical guide for appropriate dilution conditions.
	Adjust the annealing temperature in 2–3 °C increments or use a gradient PCR to find the optimal annealing temperature.
	Increase the number of amplification cycles. If currently using 25–30 cycles, increase the cycle number to 35–40.
	For complex templates like human genomic DNA, increase the initial denaturation time by 1-2 minutes and/or increase the denaturation temperature to 95 °C to overcome denaturation difficulties.
	Check concentration of input template. For complex templates like intact eukaryotic genomic DNA, 1000 genome copies may be required for amplification of difficult targets. For highly concentrated templates, such as purified plasmid, consider diluting 1:1000 to improve amplification.
	Assess DNA quality to ensure absence of PCR inhibitors in sample. If presence of inhibitors is suspected, DNA can be diluted 1:10-1:100. Alternatively, lysis and DNA purification can be performed using the GenElute™ genomic DNA miniprep kits.
	Refer to "PCR-Enhancing Additives" section of the Technical Guide to improve amplification.
	If yield is too low for downstream applications, increase the reaction volume to 50–75 µL.
Amplification of nonspecific product(s)	Raise the annealing temperature in 2–3 °C increments or use a gradient PCR to find the optimal annealing temperature. Raising the temperature improves the specificity of binding by the primers; however, it may also result in reduced binding and extension of the primers[1]. If raising the annealing temperature causes reduced yield of the specific product without eliminating side reaction products, it may be necessary to redesign the primers to improve specificity.
	Take precautions to avoid crossover contamination of PCR with both specific and nonspecific PCR products, including primer-dimer artifacts.[8]
	Titration of JumpStart™ *Taq* may be necessary to optimize PCR efficiency, especially if the reaction conditions vary from those recommended in this document. In this case, increase the concentration of JumpStart™ *Taq* by two- or four-fold. Increasing the concentration of JumpStart™ *Taq* beyond this level may inhibit PCR.
	The use of more than 5% v/v DMSO with JumpStart™ *Taq* is not recommended as it may interfere with the enzyme-antibody complex. Other co-solvents, salts, and extremes in pH can also reduce the affinity of the JumpStart™ *Taq* antibody for the *Taq* DNA Polymerase and compromise its effectiveness for hot start PCR.

References

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8. Kwok, S., and Higuchi, R., Nature 339, 237-238 (1989).

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Product Ordering

Description	Catalogue Number
Extract-N-Amp™ PCR Diluent	E8155
Extraction solution	E7526
Neutralization solution B	N3910
Dilution solution	D5688
REDExtract-N-Amp™ Tissue PCR Kit	XNAT
REDExtract-N-Amp™ Plant PCR Kit	XNAP
JumpStart™ *Taq* DNA Polymerase	D4184
Magnesium chloride solution	M1028
Deoxynucleotide (dNTP) Mix, containing 10 mM each of dATP, dCTP, dGTP, and dTTP sodium salts	D7295
Deoxynucleotide Mix (dNTP), 25 mM	D7297
Nuclease-free water	W1754
Custom ordered primers specific to gene target	OLIGO
GenElute™-E Single Spin DNA Cleanup Kit	EC600
GenElute™ PCR Clean-Up Kit	NA1020
GenElute™ Gel Extraction Kit	NA1111
Precast Agarose Gels	P6222 P5472 P6097 P5972 P5722
1 kb DNA Ladder	D0428
Water, Microbial DNA-free	MBD0025
Nuclease-Free Water, for Molecular Biology	W4502
JumpStart™ *Taq* Ready Mix	P2893
RED*Taq*® Ready Mix	P0982
Glycerol-free JumpStart™ *Taq* DNA Polymerase	D9310
DMSO	D8418
Single strand binding protein	S3917
Betaine solution	B0300
Mineral Oil	M5904