

## 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  $\geq 70$  °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

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.



### Assemble Reaction Mix

| Reagent                                 | Final Concentration | Amount per 20 $\mu$ L reaction |
|-----------------------------------------|---------------------|--------------------------------|
| SYBR Green Extract-N-Amp™ PCR Ready Mix | 1X                  | 10 $\mu$ L                     |
| Primers                                 | 0.1–0.5 $\mu$ M     | Variable                       |
| Neutralized extract                     | Variable            | 4 $\mu$ L                      |
| Nuclease-free Water                     | -                   | To 20 $\mu$ L 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  $\mu$ L 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                                    | $\infty$    |

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:<sup>1</sup>

- 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.<sup>1</sup> 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.<sup>1</sup>

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.<sup>2</sup>

### 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.

|           |                         |        |               |
|-----------|-------------------------|--------|---------------|
| 40 cycles | Initial denaturation    | 94 °C  | 3 minutes     |
|           | Denaturation            | 94 °C  | 3 seconds     |
|           | Annealing/<br>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/<br>Saliva/Buccal<br>swab | 50:50 mixture of Extraction and Neutralization B Solutions or with Extract-N-Amp™ PCR Diluent |

### Recommended MgCl<sub>2</sub> Concentration

Because JumpStart™ *Taq* DNA Polymerase is a magnesium ion-dependent enzyme, the optimal concentrations of template DNA, primers, and MgCl<sub>2</sub> will be target-dependent. The optimal MgCl<sub>2</sub> concentration is also dependent upon the intended application. Reactions can be supplemented with up to 0.5 mM of additional MgCl<sub>2</sub> 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<br>(10-100 µg/mL)                         | <i>Taq</i> DNA polymerase stabilization <sup>2</sup>                                                                  |
| Formamide<br>(1.25-10%)                       | Increases specificity in G/C rich regions <sup>3</sup>                                                                |
| DMSO<br>(Up to 5%)                            | Accelerates strand renaturation <sup>4</sup><br>Nucleic acid thermal stability against depurination <sup>4</sup>      |
| Glycerol<br>(Up to 10%)                       | Increases thermal stability of the polymerase and lowers the temperature necessary for strand separation <sup>4</sup> |
| Ammonium sulfate<br>(15-30 mM)                | Affects the denaturing and annealing temperatures of the DNA <sup>5</sup>                                             |
| Single strand binding protein<br>(0.7-1.5 µg) | Inhibits formation of secondary structures, improving fidelity and <i>Taq</i> processivity <sup>6</sup>               |
| Betaine<br>(0.8-1.6 M)                        | Reduces base pair composition dependence of DNA melting <sup>7</sup>                                                  |

## 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 <a href="#">Recommended <math>MgCl_2</math> Concentration</a> 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 <a href="#">Technical guide</a> 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.                                                                                                                                                                                                    |
| Amplification of nonspecific product(s) | 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 <a href="#">Technical Guide</a> to improve amplification.                                                                                                                                                                                                                                                                                                                                                                                    |
|                                         | If yield is too low for downstream applications, increase the reaction volume to 50–75 µL.                                                                                                                                                                                                                                                                                                                                                                                                     |
|                                         | 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 <sup>1</sup> . 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. |
| Amplification of nonspecific product(s) | Take precautions to avoid crossover contamination of PCR with both specific and nonspecific PCR products, including primer-dimer artifacts. <sup>8</sup>                                                                                                                                                                                                                                                                                                                                       |
|                                         | 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

1. Green MR, Sambrook J., Cold Spring Harb Protoc. 6, 436-456(2019).
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3. Sarkar, G., et al., Nucleic Acids Res., 18, 7465 (1990).
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6. Chou, Q., Nucleic Acids Res., 20, 4371 (1992).
7. Rees, W. A., et al., Biochemistry, 32, 137-144 (1993).
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            |
| REExtract-N-Amp™ Tissue PCR Kit                                                              | XNAT             |
| REExtract-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           |
|                                                                                              | P6222            |
|                                                                                              | P5472            |
| Precast Agarose Gels                                                                         | 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            |
| REDTaq® Ready Mix                                                                            | P0982            |
| Glycerol-free JumpStart™ Taq DNA Polymerase                                                  | D9310            |
| DMSO                                                                                         | D8418            |
| Single strand binding protein                                                                | S3917            |
| Betaine solution                                                                             | B0300            |
| Mineral Oil                                                                                  | M5904            |

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