Product Information

SYBR Green Extract-N-Amp™ Tissue PCR Kit

XNATG, XNATRG

Product Description

The SYBR Green Extract-N-Amp™ Tissue PCR Kit for direct PCR contains the reagents needed for rapid extraction, amplification and detection of genomic DNA from mouse tails and other animal tissues, buccal swabs, hair shafts, and saliva. DNA is rapidly extracted from a tissue by incubating the sample with a mixture of the Extraction Solution and the Tissue Preparation Solution at room temperature for 10 minutes. There is no need for freezing tissue in liquid nitrogen, mechanical disruption, organic extraction, column purification or precipitation of the DNA. After a 3-minute heat denaturing step, an equal volume of Neutralization Solution B is added to the extract to neutralize inhibitory substances and 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* 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.

		XNATG	**XNATRG**
Reagents Provided	**Cat. No.**	100 extractions 100 amplifications	1,000 extractions 1,000 amplifications
Extraction Solution	E7526	24 mL	240 mL
Neutralization Solution B	N3910	24 mL	240 mL
Tissue Preparation Solution	T3073	3.0 mL	30 mL
Extract-N-Amp™ SYBR Green PCR ReadyMix. This is a 2X real-time PCR reaction mix containing SYBR Green, buffer, salts, dNTPs, *Taq* polymerase and JumpStart™ *Taq* antibody.	S4320	1.2 mL	12 mL

Reagents and Equipment Required (Not provided)

- Microcentrifuge tubes (1.5 or 2 mL) or multi-well plate for extractions
- Small dissecting scissors or scalpel
- Forceps (small to medium in size)
- Buccal swab (Sterile foam tipped applicator, Cat. No. WHAWB100032)
- Tubes or plate for PCR
- Heat block or thermal cycler at 95 °C
- PCR Primers, Cat. No. OLIGO
- Plate-based Thermal cycler capable of real-time SYBR Green detection.
- Reference dye, if required for real-time thermal cycler
- Water, PCR grade, Cat. No. W1754

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 of Kit Components

The Extraction, Tissue Preparation & Neutralization 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 & 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 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 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 (Cat. No. E8155). 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. See Real-Time PCR amplification.

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 a 50:50 mixture of Extraction and Neutralization Solutions or Extract-N-Amp™ PCR Diluent in a 20 µL PCR reaction. See Real-Time PCR amplification, Step 1. Do not use water as a negative control.

Procedure

All steps are carried out at room temperature unless otherwise noted. The Extraction, Neutralization and Tissue Preparation solutions should be completely thawed, brought to room temperature and gently mixed before use. The Extract-N-Amp™ SYBR Green PCR ReadyMix should be thawed, mixed well, but kept on ice and protected from light until needed.

DNA Extraction from Mouse Tails, Animal Tissues, Hair, or Saliva

1. Pipette 100 µL of Extraction Solution into a microcentrifuge tube or well of a multi-well plate. Add 25 µL of Tissue Preparation Solution to the tube or well and pipette up and down to mix.

   Note: If several extractions will be performed, sufficient volumes of Extraction and Tissue Preparation Solutions may be pre-mixed in a ratio of 4:1 up to 2 hours before use.

2. **For fresh or frozen mouse tails**

   Rinse the scissors or scalpel and forceps in 70% ethanol prior to use and between different samples. Place a 0.5-1 cm piece of mouse tail tip, cut end down, into the Extraction/Tissue Preparation Solution mixture. Ensure that the cut end of the mouse tail is completely submerged in the solution.

   Note: Frozen tails may be cut and used as they thaw. For fresh mouse tails, perform extractions within 30 minutes of snipping the tail.

   For animal tissues

   Rinse the scissors or scalpel and forceps in 70% ethanol prior to use and between different samples. Place a 2-10 mg piece of tissue into the Extraction/Tissue Preparation Solution mixture. Ensure that the tissue is completely submerged in the solution.

   For hair shafts

   Rinse the scissors and forceps in 70% ethanol prior to use and between different samples. Trim excess off of the hair shaft, leaving the root and place sample (root end down) into the Extraction/Tissue Preparation Solution mixture. Only one hair shaft, with root, is required per extraction.

For saliva

Pipette 10 µL of saliva into the Extraction/Tissue Preparation Solution mixture. Mix thoroughly by vortexing or pipetting.

3. Incubate sample at room temperature for 10 minutes.
4. Incubate sample at 95 °C for 3 minutes. Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.

   Note: This is a critical step! 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. Additional time needed must be determined empirically.
5. Add 100 µL of Neutralization Solution B to heated extract and mix by vortexing.
6. Store the neutralized tissue extract at 4 °C or use immediately in Real-Time PCR amplification.

   Note: For long term storage, remove the undigested tissue or transfer the extracts to new tubes or wells. Extracts may now be stored at 4 °C for at least 6 months without notable loss in most cases.

DNA Extraction for Buccal Swabs

1. Collect buccal cells on swab and allow the swab to dry. Drying time is approximately 10 to 15 minutes.

   Note: Due to the low volume of solution used for DNA extraction, a foam tipped swab should be used. Swabs with fibrous tips, such as cotton or Dacron, should be avoided because the solution cannot be recovered efficiently.
2. Pipette 200 µL of Extraction Solution into a 1.5 mL microcentrifuge tube. Add 25 µL of Tissue Preparation Solution to the tube and pipette up and down to mix.

   Note: If several extractions will be performed, sufficient volumes of Extraction and Tissue Preparation Solutions may be pre-mixed in a ratio of 8:1 up to 2 hours before use.
3. Place dried buccal swab into the solution and incubate at room temperature for 1 minute.
4. Twirl swab in solution 10 times and then remove excess solution from the swab into the tube by twirling swab firmly against the side of the tube. Discard the swab. Close the tube and vortex briefly.
5. Incubate sample at room temperature for 10 minutes.
6. Incubate sample at 95 °C for 3 minutes.

   Note: This is a critical step! 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. Additional time needed must be determined empirically.
7. Add 200 µL of Neutralization Solution B to heated extract and mix by vortexing.
8. Store the neutralized extract at 4 °C, or use immediately in Real-Time PCR amplification (see process below).

   Note: Extracts may be stored at 4 °C for at least 6 months without notable loss in most cases.

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 tissues or other starting materials, PCR may be inhibited by secondary metabolites in the extract such that little or no detectable PCR product is obtained. 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 in many 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 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
Extract-N-Amp™ SYBR Green PCR ReadyMix	10 µL
Water, PCR Grade	Variable
Forward Primer	Variable
Reverse Primer	Variable
Reference dye*	Variable*
Neutralized Extract	4 µL
Total Volume	20 µL

   * If required for real-time PCR instrument.

   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. Generate a volume in excess of the amount required to account for measuring losses. After determining the appropriate volume for all reagents, combine and gently vortex to create master-mix. Aliquot 16 µL of master mix and 4 µL of extract per reaction.

2. **Mix & Spin Down**
   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	30 seconds	
Annealing	45 to 68 °C	30 seconds	
Extension	72 °C	10 seconds to 1 minute (1 min/kb)	30-40

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 product on a 2% Agarose-TBE buffered gel.

References

1. Ririe KM, Rasmussen RP, Wittwer CT, Product differentiation by analysis of DNA melting curves during the polymerase chain reaction, Anal. Biochem. 1997 Feb 15;245(2):154-60.

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.
	95 °C heat step for DNA extraction from sample was inadequate	Verify that extracts were heated in a water bath or a block that fits the contour of tubes, and that the bath or block was at 95 °C for the entire incubation time. Use of other heating devices, such as an oven, will require longer incubation times that must be determined empirically.
	Extract was stored without removing tail snip or tissue.	Remove undigested tail snip or tissue before storing extracts at 4 °C. Extracted DNA may not be stable in the presence of undigested tissue.
	PCR reaction may be inhibited due to secondary metabolites in the extract.	Dilute the extract 5-, 10-, or 20-fold with a 50:50 mix of Extraction and Neutralization Solutions or Extract-N-Amp™ PCR Diluent (Cat. No. E 8155) and repeat PCR with 4 µL of diluted extract.

Problem	Possible Cause	Solution
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 Tm of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer Tm 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 step in the cycling program at a temperature slightly below the Tm of the desired PCR product (approximately 3 °C) where fluorescence is read to avoid detection of primer-dimers.
	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.

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