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Product Information

REDExtract-N-Amp™ Tissue PCR Kit

Catalog Numbers **XNATS, XNAT, XNATR**

TECHNICAL BULLETIN

Product Description

The REDExtract-N-Amp Tissue PCR Kit contains all the reagents needed to rapidly extract and amplify genomic DNA from mouse tails and other animal tissues, buccal swabs, hair shafts, and saliva. Briefly, the DNA is released from the starting material 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 mechanical disruption, organic extraction, column purification, or precipitation of the DNA.

After adding Neutralization Solution B, the extract is ready for PCR. An aliquot of the neutralized extract is then combined with the REDExtract-N-Amp PCR Reaction Mix and user-provided PCR primers to amplify target DNA. The REDExtract-N-Amp PCR Reaction Mix is a 2x reaction mixture containing buffer, salts, dNTPs, and *Taq* polymerase. It is optimized specifically for use with the extraction reagents. It also contains the JumpStart™ *Taq* antibody for hot start PCR to enhance specificity and the REDTaq® dye to allow direct loading of the PCR product onto an agarose gel.

Reagents Provided	Catalog Number	XNATS 10 Preps, 10 PCRs	XNAT 100 Preps, 100 PCRs	XNATR 1000 Preps, 1000 PCRs
Extraction Solution	E7526	2.5 ml	24 ml	240 ml
Tissue Preparation Solution	T3073	0.3 ml	3 ml	30 ml
Neutralization Solution B	N3910	2.5 ml	24 ml	240 ml
REDExtract-N-Amp PCR Reaction Mix, **This is a 2x PCR reaction mix** containing buffer, salts, dNTPs, *Taq* polymerase, REDTaq dye, and JumpStart *Taq* antibody.	R4775	0.15 ml	1.2 ml	12 ml

Reagents and Equipment required, not provided

- Microcentrifuge tubes (1.5 or 2 ml) or multiwell plate for extractions (200 µl minimal well volume)
- Scissors, micro-dissecting, Catalog No. Z265985
- Forceps (small to medium in size)
- Buccal swab (Sterile foam tipped applicator, Catalog Number A9601
- Sample collection card - Whatman® FTA® collection products, Catalog Number Z719838
- Tubes or plate for PCR
- Heat block or thermal cycler at 95 °C
- PCR Primers
- Thermal cycler
- Water, PCR Reagent, Catalog Number W1754

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

The REDExtract-N-Amp Tissue PCR Kit can be stored at 2 to 8 °C for up to 3 weeks. For long-term storage, greater than 3 weeks, –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 from Mouse Tails, Animal Tissues, Hair, or Saliva

1. Pipette 100 µL of Extraction Solution into a microcentrifuge tube or well of a multiwell 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.

2a. **For Fresh or Frozen Mouse Tails:** Rinse the scissors 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 solution. Mix thoroughly by vortexing or pipetting. Ensure the mouse tail is in the solution.
   Note: For fresh mouse tails, perform extractions within 30 minutes of snipping the tail.

2b. **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 solution. Mix thoroughly by vortexing or pipetting. Ensure the tissue is in the solution.

2c. **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 solution. Only one hair shaft, with root, is required per extraction.

2d. **For Saliva:** Pipette 10 µL of saliva into the solution. Mix thoroughly by vortexing or pipetting.

2e. **For Saliva Dried on a Card:** Pipette 50 µL of saliva onto a collection card and allow the card to dry. Rinse the hole punch in 70% ethanol prior to use and between different samples. Punch a disk (preferably 1/8 inch or 3 mm) out of the card from the area with the dried saliva sample. Place disk into the solution. Tap tube or plate on hard surface to ensure disk is in the solution for incubation period.

3. Incubate sample at room temperature for 10 minutes.
4. Incubate sample at 95 °C for 3 minutes.
   Note: Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.
5. Add 100 µL of Neutralization Solution B to sample and mix by vortexing.
6. Store the neutralized tissue extract at 4 °C or use immediately in PCR. Continue with Section C, step 1.
   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.

B. 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 can not 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.
7. Add 200 µL of Neutralization Solution B to sample and mix by vortexing.
8. Store the neutralized extract at 4 °C or use immediately in PCR. Continue with Section C, step 1.
   Note: Extracts may be stored at 4 °C for at least 6 months without notable loss in most cases.

PCR amplification
The REDExtract-N-Amp PCR Reaction Mix 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 approximately 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
REDExtract-N-Amp PCR Reaction Mix	10 μL
Forward primer	y μL
Reverse primer	y μL
Tissue extract	4 μL*
Total volume	20 μL

*Note: The REDExtract-N-Amp PCR Reaction Mix is formulated to compensate for components in the Extraction, Tissue Preparation, and Neutralization Solutions. If less than 4 μL of tissue extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction:Neutralization B Solutions to bring the volume of tissue extract up to 4 μL.

2. Mix gently.
3. For thermal cyclers without a heated lid, add 20 μL of mineral oil on top of the mixture in each tube to prevent evaporation.
4. Perform thermal cycling. 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	

5. The amplified DNA can be loaded directly onto an agarose gel after the PCR is completed. It is not necessary to add a separate loading buffer/tracking dye.
   Note: PCR products can be purified, if desired, for downstream applications such as sequencing with the GenElute™ PCR Clean-Up Kit, Catalog Number NA1020.

References

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10. Saiki, R., PCR Technology: Principles and Applications for DNA Amplification, Stockton, New York (1989).

Related Products	Catalog Number
Ethanol	E7148; E7023; 45 9836
Forceps, micro-dissecting	F4267
PCR Marker	P 9577
PCR microtubes	Z374873; Z374962; Z374881
PCR multiwell plates	Z374903
Precast Agarose Gels	P6097
Sealing mats & tapes	Z374938; A2350
TBE Buffer	T4415, T6400, T9525

Troubleshooting Guide

Problem	Cause	Solution
Little or no PCR product is detected.	PCR reaction may be inhibited due to contaminants in the tissue extract.	Dilute the tissue extract with a 50:50 mix of Extraction and Neutralization 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 tissue extract.
	Extraction is insufficient.	Incubate samples at 55 °C for 10 minutes instead of room temperature.
	A PCR component may be missing or degraded.	Run a positive control to ensure 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 in 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 in 1 °C increments.
	The denaturation time may be too long or too short.	Optimize the denaturation time by increasing or decreasing it in 10 second increments.
	The extension time may be too short.	Increase the extension time in 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, Catalog Number B0300, 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 REDExtract-N-Amp PCR Reaction Mix. 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 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.

Troubleshooting Guide (continued)

Negative control shows a PCR product or "false positive" result.	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.
Tissue is not digested after incubations.	Tissue is not expected to be completely digested.	The REDExtract-N-Amp Tissue PCR Kit does not require the tissue to be completely digested. Sufficient DNA is released for PCR without completely digesting the tissue.
Buccal swab absorbed all the solution.	The recommended type of swab was not used.	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 can not be recovered efficiently.

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