

Product Information

Automated Protocol for Extract-N-Amp™ Tissue PCR Kits Using the Sciclone ALH 3000 Workstation (Caliper Life Sciences)

Extract-N-Amp Tissue Product Codes XNATR and XNAT2R

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Automation Guide

I. Description

The Extract-N-Amp™ Tissue PCR kit has been developed for use as a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA from mouse tails and other animal tissues in a 96-well format. The Extract-N-Amp Tissue PCR kits provide a novel DNA extraction system, eliminating the need for long enzymatic digestions and homogenization steps that are not amenable to automation. Included in the kit is a specially formulated Extract-N-Amp PCR ReadyMix™ that is a 2x reaction mixture of buffer, salts, dNTPs, and *Taq* polymerase. It also contains Sigma's antibody mediated hot start polymerase, JumpStart™, for highly specific amplification of genomic DNA directly from the extract. There is a second formulation of the ReadyMix, REExtract-N-Amp PCR ReadyMix that contains an inert tracking dye for convenient direct loading of the PCR reactions onto an agarose gel for analysis.

The automated method created and validated for use on the Sciclone ALH 3000 Liquid Handling Workstation from Caliper Life Sciences provides a walk-away protocol for all aspects of the Extract-N-Amp Tissue PCR kit.

Extraction and amplification of genomic DNA from animal tissues is accomplished in 4 easy steps:

1. The Extraction and Tissue Preparation Solution mixture is added to tissue samples and incubated at room temperature for 10 minutes.
2. Extracts are incubated for 15 minutes at 85 °C.
3. A Neutralization Solution is added to the extract. Once the Neutralization Solution has been added, extracts can be stored at 4 °C for at least 6 months.
4. PCR reactions are set up using 4 µl of the extracts.

In just 45 minutes, the Sciclone ALH 3000 can complete the extraction and PCR reaction setup for 96 tissue samples.

II. Product Components

Reagents Provided	Product Code	Extract-N-Amp Tissue XNAT2R	REExtract-N-Amp Tissue XNATR
	Package Size	1000 extractions 1000 amplifications	1000 extractions 1000 amplifications
Extraction Solution	E 7526	240 ml	240 ml
Tissue Preparation Solution	T 3073	30 ml	30 ml
Neutralization Solution B	N 3910	240 ml	240 ml
Extract-N-Amp PCR Ready Mix or REExtract- N-Amp PCR Ready Mix	E 3004 (for XNAT2R) R 4775 (for XNATR)	12 ml	12 ml

III. Storage

The Extract-N-Amp Tissue PCR Kits can be stored at 2-8 °C for up to 3 weeks. For long-term storage, store at –20 °C. Do not store in a frost-free freezer.

IV. Materials to Be Supplied by the User

1. Animal Tissues
2. Small dissecting scissors
3. Forceps (small to medium in size)
4. Primers for genes of interest
5. Molecular biology grade water (Sigma, W 4502)
6. 96-well PCR plates, with full skirt (Sigma, P 4616)
7. 96-well PCR plates, with half skirt (ABgene, AB-1100)
8. Lid, universal (Fisher, 07200694)
9. Ultra clear cap strip (ABgene, AB-0866)
10. Corning plate holder (Corning, 6525)
11. Sealing film, SealPlate (Sigma, Z36,965-9)
12. Micro centrifuge tubes (1.5 ml, 2 ml screw cap)
13. 24-position Eppendorf IsoTherm System (Fisher, 05-405-22)
14. 12 column reagent reservoir with low profile (Innovative Microplates, S30028)
15. 96 well reservoir with low profile and pyramidal bottom (Innovative Microplates, S30018)
16. (Optional) High profile 12 column reagent reservoir (Innovative Microplates, S30019)
17. (Optional) High profile 96-well reservoir with pyramidal bottom (Innovative Microplates, S30014)
18. Thermal Cycler
19. Thermometer (Fisher, 15-077-26)

V. Tissue Preparation

For Fresh or Frozen Mouse Tails:

1. Rinse scissors and forceps in 70% ethanol prior to use and between different samples. Place a 0.3 – 0.4 cm piece of mouse tail tip (cut end down) into a 96-well PCR plate ensuring that each sample is centered down into the bottom of each well.
2. Chill the plate at 2-8 °C until needed.

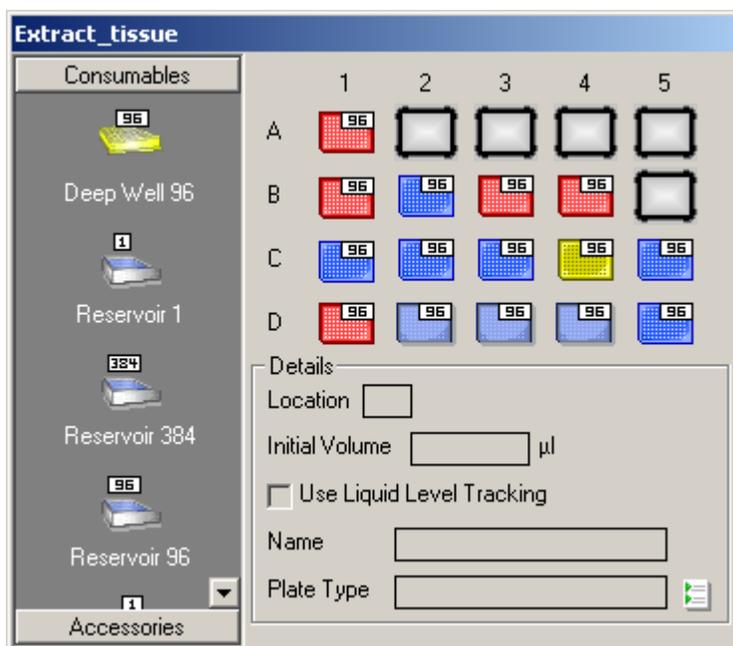
Other Animal Tissues:

1. Rinse scissors and forceps in 70% ethanol prior to use and between different samples. Place a 4 – 6 mg piece of tissue into a 96-well PCR plate ensuring that each sample is centered down into the bottom of each well.
2. Chill the plate at 2-8 °C until needed.

VI. Instrument Requirements for the Sciclone ALH 3000 Workstation

Part Description	Qty	Ordering Information
Deck Mounted Shaker	1	Contact Caliper
High Temperature Control Device	1	Contact Caliper
96 well PCR Plate Adapter	1	Contact Caliper
96-channel High Volume Head	1	Contact Caliper
Z-8™ Pipettor	1	Contact Caliper
Gripper	1	Contact Caliper
I/O Box	1	Contact Caliper
Deck Locator	8	Contact Caliper
Tip Box Locator	5	# 76523 (Caliper)
100 µl Disposable Tip Box	2	# 66670 (Caliper)
80 µl Barrier Tip Box	2	# 68759 (Caliper)
200 µl Disposable Tip Box	1	# 56362 (Caliper)

VII. Deck Setup



Deck Position	Equipment
A1	200 µl Tip box
B1	100 µl Tip box
B2	Lid position for tissue sample plate
B3	80 µl Tip box, Barrier Tips
B4	80 µl Tip box, Barrier Tips
C1	96-well PCR plate with full skirt for transferring neutralized tissue extracts for long-term storage
C2	96-well PCR plate with full skirt containing tissue samples (with Lid)
C3	96-well PCR plate with half skirt for PCR reaction setup (seated into a plate holder)
C4	24 position Eppendorf IsoTherm system
C5	PCR plate adapter on temperature control device
D1	100 µl Tip box
D2	96-well reservoir for Neutralization Solution
D3	12-column reservoir for PCR master mix
D4	96-well reservoir for the mixture of Extraction and Tissue Preparation Solution
D5	Shaker

VIII. Temperature Control Device Setup

Set the temperature control device to the maximum setting of 100 °C with an offset of 15 °C (refer to the User's Manual). Place a PCR plate in the device and measure the temperature inside the wells using thermometer probes. Verify that the temperature in the wells is at a minimum of 85 °C after 3 minutes. Approximately one hour prior to running the automated method, turn on the temperature control device and verify that the temperature display on the controller has reached the desired reading.

IX. Reagent Preparation

1. *Extraction and Tissue Preparation Solution Mixture*: Pre-mix the Extraction and Tissue Preparation Solutions at a ratio of 4:1. This solution can be stored for up to 2 hours before use. To process a single plate of 96 samples, add 15 ml of the mixture to the 96-well reservoir located at position D4. If it is desired to process more than 12 plates of samples, the high-profile reservoir (S30014) is required.
2. *Neutralization Solution*: To process a single plate of 96 samples, add 15 ml of Neutralization Solution to the 96-well reservoir located at position D2. If it is desired to process more than 12 plates of samples, the high-profile reservoir (S30014) is required.
3. *PCR Master Mix*: The Extract-N-Amp Tissue PCR ReadyMix is a 2x reaction mixture containing buffer, salts, dNTPs, and Taq polymerase. To prepare a Master mix add water and primers (forward and reverse) to the Extract-N-Amp Tissue PCR ReadyMix as described in table below.

	Water	PCR Mix	Forward Primer	Reverse Primer
Stock		E 3004	100 μ M	100 μ M
Working (2.7 ml)	0.9 ml	1.8 ml	16 μ l	16 μ l

To set up 20 μ l PCR reactions in one 96-well plate, add 2.7 ml PCR master mix to the second column of the reservoir located at position D3. If setting up more than 3 plates of samples for PCR, it will be necessary to use reservoir S30019.

4. *No-template Control (optional)*: Add water to four 2-ml screw cap tubes and place in column 5 of the 24-position tube rack located at position C4.
5. *DNA Controls (optional)*: Prepare genomic DNA controls for quantification of tissue DNA extracts. Prepare 4 tubes containing 10 ng/ μ l, 5 ng/ μ l, 1.25 ng/ μ l, and 0.31 ng/ μ l samples of genomic DNA and place in column 4 of the 24-position tube rack located at position C4.

X. Automated Method Description

This overview describes the general liquid handling steps required to execute the automated Extract-N-Amp Tissue PCR method and can be customized to a variety of applications. To customize applications, see Section XII.

A. General Procedures:

1. Turn on temperature control device.
2. Turn the plate shaker module controller clockwise a third past the first black line. If using the Teleshake v1.2 software, use a setting of 750 rpm.
3. Set up the deck layout by placing the tip boxes, plates, tube racks and reservoirs at the appropriate positions on the deck as described in section VII.
4. Add reagents to the appropriate reservoirs as described in section IX.
5. Run the method using Sciclone Software Version 3.2.
6. At the completion of the method, place cap strips onto the PCR plate, vortex to mix the solution and briefly centrifuge. The PCR plate is now ready to be placed into a thermal cycler.
7. Seal the PCR plate containing tissue extracts with a sealing film. Tissue extracts can be stored for up to 6 months at 4 $^{\circ}$ C.

B. Sciclone Methods:

Two methods have been created for this kit:

Extract-N-Amp_Tissue: Performs all of the steps necessary to extract DNA from 96 tissue samples and PCR reaction setup.

PCR_Setup: Performs PCR reaction setup for 96 samples using a master mix. This method may be used if it is desired to perform additional amplification experiments from tissue extracts.

Extract-N-Amp Tissue: Method Overview

Below is a summary of the Extract-N-Amp Tissue automated method. For complete program details download the automation program at www.sigmaaldrich.com/automation

1. The Sciclone head movement speed is set to 80%
2. The lid is removed from the 96-well PCR plate containing tissue samples
3. Disposable tips are loaded onto the 96-channel high volume head

4. The Extraction and Tissue Preparation solution is aspirated from the appropriate reservoir
5. The mixture is dispensed to the extraction plate containing the tissue samples
6. Used tips are disposed
7. The plate is moved to the shaker and mixed for 30 seconds
8. The plate is incubated for 10 minutes at room temperature
9. The plate is moved to the temperature control device and incubated at 85 °C for 15 minutes
10. Following the incubation period, the plate is moved back to position C2 on the Sciclone deck
11. Disposable tips are loaded onto the 96-channel high volume head
12. 50 µl of neutralization solution is aspirated from a reservoir by the 96 channel high volume head.
13. Neutralization solution is dispensed into the extraction plate
14. Samples are mixed using pipette-mixing for 8 cycles
15. Used tips are discarded
16. The plate is moved to the shaker and mixed for 30 seconds
17. The plate is moved back to position C2 on the Sciclone deck
18. The *PCR_Setup Method* is called up and performs the steps necessary to setup PCR reactions (overview of the method below)
19. Disposable tips are loaded onto the 96-channel high volume head
20. Tissue extracts are aspirated from the extraction plate and dispensed into a clean PCR plate located at position C1
21. The tips are discarded
22. Place the lid on the plate

PCR Set Up: Method Overview

Below is a summary of the PCR Setup method. For complete program details, download automation program from www.sigmaaldrich.com/automation

1. The Sciclone head movement speed is set to 80%
2. Barrier disposable tips are loaded onto the Z-8 dispense head
3. The PCR master mix is aspirated from the 12-column reservoir. Acting like a bulk dispenser, the Z-8 head aspirates enough reagent to dispense into a quarter of the plate
4. Steps 3 is repeated until the PCR master mix to all 96 wells of the PCR plate
5. The tips are discarded
6. A new set of barrier tips are loaded onto the Z-8 dispense head
7. Tissue extracts are aspirated from the multiwell plate containing tissue extracts prepared during the preceding method and dispensed into the PCR amplification plate
8. The tips are discarded
9. A loop has been created, repeating steps 7 and 8 in order to pipette all samples into the PCR amplification plate
10. Barrier tips are loaded onto the Z-8 dispense head
11. Control DNA samples or water are aspirated from the microfuge tubes located at position C4
12. The control DNA samples or water is dispensed to the last column of the PCR amplification plate
13. The tips are discarded

XI. Recommended Parameters for PCR Amplification:

Step	Temperature	Time	Cycles
Initial Denaturation	94-96 °C	3 minutes	1
Denaturation	94-96 °C	0.5-1 minutes	
Annealing	45-68 °C	0.5-1 minutes	30-40
Extension	72 °C	1-2 minutes (~1 kb/min)	
Final Extension	72 °C	10 minutes	1
Hold	4 °C	Indefinitely	

XII. Method Customization

PCR setup only

Tissue extracts may be subjected to additional amplifications. The PCR Setup method described in Section X may be used for this purpose.

Use of a different PCR plate

The automated method was created using the 96-well PCR amplification plates with half skirt from Abgene for the extraction steps. Other PCR plates may be used in this method, but may require the creation of a new labware in the Sciclone software. If a different PCR plate is used, the tip touch in command lines 12, 21, 30, 38-42, and 61-63 of the *PCR_Setup* method may need to be adjusted. This tip touching is a critical step for the addition of the low volume of tissue DNA extract to the reaction mixture.

PCR setup using multiple primer sets

To amplify genomic DNA from the 96 tissue extracts with different primer sets, primers can be added to microfuge tubes and placed on the 24-position tube racks. Additional steps will need to be added to the automated program after command line 32 in the *PCR_Setup* method to account for the primer addition.

Transfer of tissue extracts to a new plate

For long-term storage of tissue extracts it is desirable to transfer them to a new plate. Because the size of tissue samples may vary from mouse-tails, it may be necessary to adjust the height of aspiration in command line 39 of the *Extract-N-Amp_Tissue* method to avoid clogging of the pipet tips with tissue samples. In some instances, manual transfer of the extracts to a new plate may be required. If this is the case, the command lines 37-43 of the *Extract-N-Amp_Tissue* method are deleted.

XIII. Performance Characteristics

Automated Method for the Extract-N-Amp PCR Analysis of Mouse Tails Samples

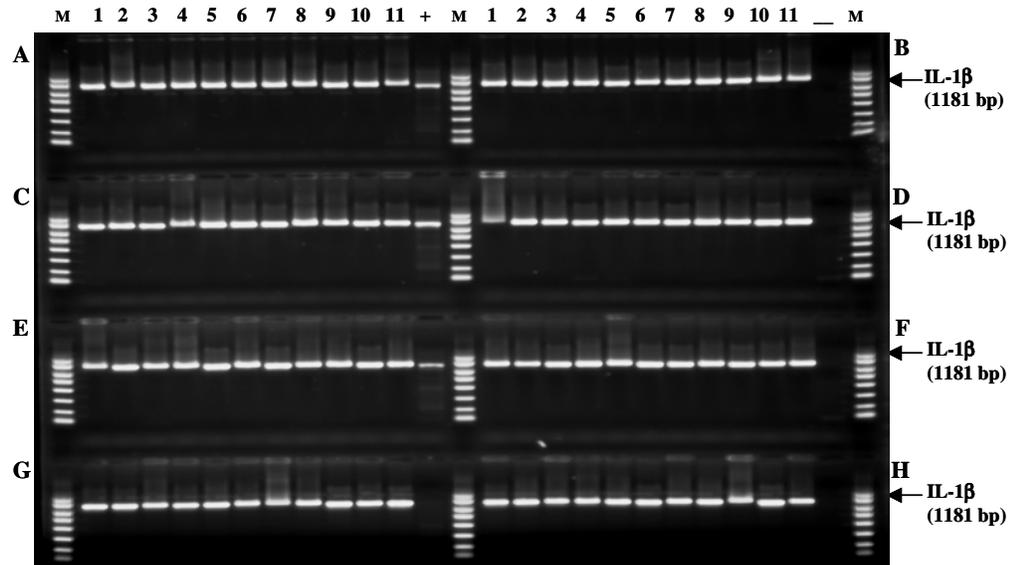


Figure 1. Agarose gel analysis of 96 PCR samples. DNA was extracted from 88 samples of mouse-tails (0.3 – 0.4 cm) using the automated Extract-N-Amp Tissue PCR procedure on the Sciclone ALH 3000 workstation. Amplification of the 1181bp of the IL-1 β gene followed using 4 μ l of extracted template or 4 μ l of human genomic DNA controls in a 20 μ l PCR reaction incorporating the 2X PCR ReadyMix. 6 μ l of each reaction was analyzed on a 1% Agarose gel.

Cross-Contamination Analysis

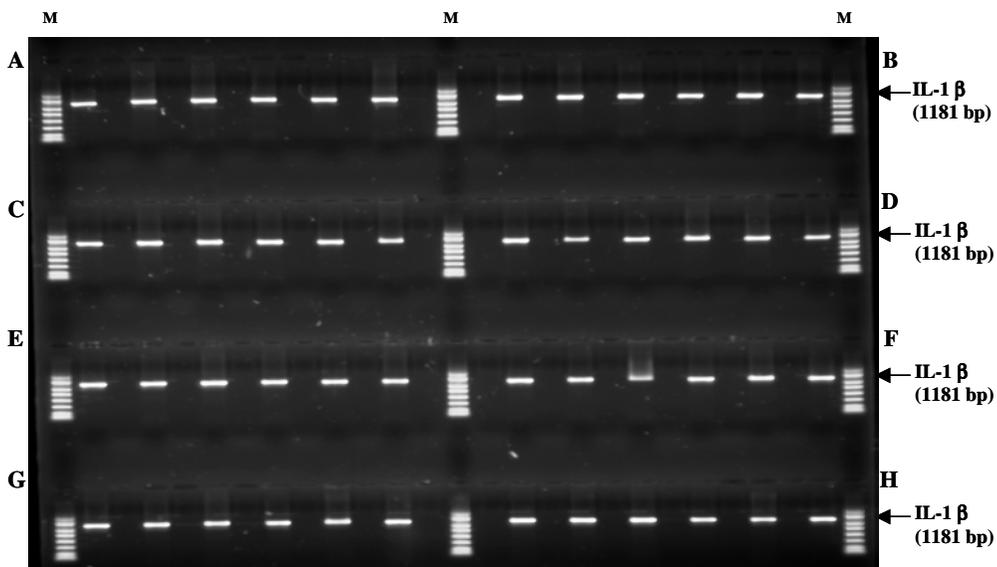


Figure 2. Cross-contamination. Mouse-tails were placed in alternating wells of the extraction plate. The extraction plate was processed using the automated Extract-N-Amp Tissue PCR procedure on the Sciclone ALH 3000 workstation. All samples were amplified and 6 μ l of the resultant products were electrophoresed on a 1% agarose gel. No PCR products were detected in the wells without tissue samples.

Multiple Tissue Samples

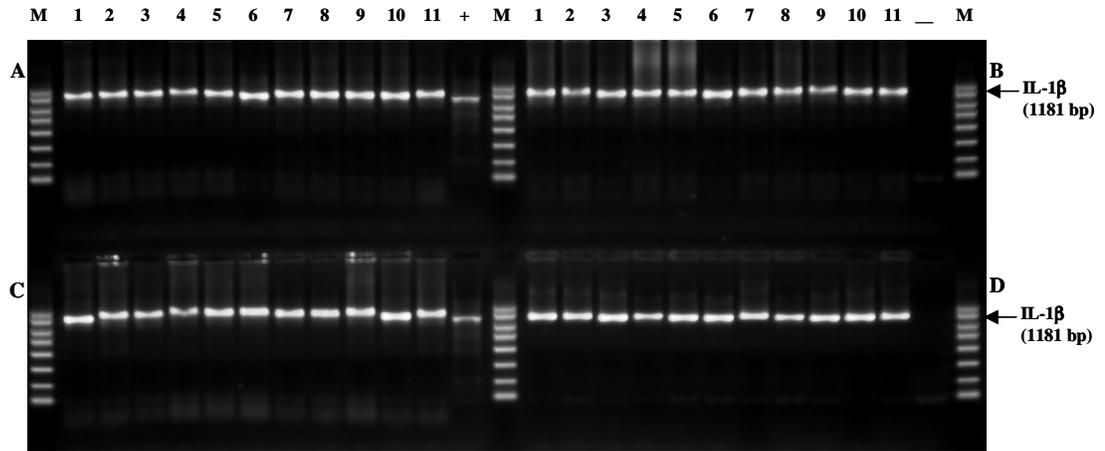


Figure 3: Analysis of Multiple Tissue Samples. DNA was extracted from mouse liver, mouse kidney, mouse pancreas, and mouse-tails using the automated Extract-N-Amp Tissue PCR procedure on the Sciclone ALH 3000 workstation. Amplification of the 1181 bp fragment of the IL-1 β gene followed using 4 μ l of extracted template DNA or 4 μ L of human genomic DNA controls in a 20 μ l PCR reaction incorporating the 2X PCR ReadyMix. 6 μ l of each reaction was loaded on a 1% agarose gel, with mouse liver samples in lanes A1-A11, mouse kidney samples in lanes B1-B11, mouse pancreas samples in lanes C1-C11, and mouse-tails in lanes D1-D11.

XIV. Troubleshooting

Problem	Cause	Solution
Little or no PCR product is detected.	A PCR component is missing or degraded.	Run a positive control to ensure components are functioning.
	No tissue extract is added to the PCR reactions.	Check the performance of liquid handler. Prime the system if needed. Adjust the aspiration distance of the pipettors in the extraction plate.
	PCR reaction is inhibited due to contaminants in the tissue extract.	Use less extract or dilute the extract with 50:50 mix of Extraction and Neutralization Solutions and repeat PCR.
	PCR reaction is inhibited due to the presence of a precipitate that may form in the tissue extract.	Centrifuge the plate containing tissue extracts before adding the extracts to PCR amplification plate.
	The mixing of Neutralization Solution with tissue DNA extract is not sufficient due to inefficient mixing by the Sciclone and/or the clogging of the pipet tip by the tissue.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps. Decrease the aspiration distance of the pipet tips in the mixing steps to avoid sucking up the tissue by the pipettors.
	Genomic DNA is sheared when mix the solution with the pipettor.	Reduce the aspiration and dispensing speed and/or cycle times in the mixing steps. It is critical for amplifying the large genomic DNA fragments.
	Too few cycles are performed.	Increase the number of cycles (5-10 additional cycles at a time).
	Others	Refer to the Technical Bulletin of Extract-N-Amp™ Tissue PC Kits.
Negative control shows a PCR product or “false positive” results are obtained.	Reagents are contaminated.	Use new labware and new batch of reagents. Test a reagent blank without DNA template to determine if the reagents used in extraction or PCR are contaminated.

XV. Contact Information

Technical Service Help
(800) 325-5832
www.techserv@sial.com

Customer Service Help
(800) 325-3010
(800) 588-9160
www.sigma-aldrich.com/order

JumpStart and JumpStart *Taq* Antibody are licensed under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries.