3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Automated Protocol for Extract-N-Amp™ Tissue PCR Kits

Using the Eppendorf® epMotion® 5075 VAC Automated Pipetting System

Description

The Extract-N-Amp™ Tissue PCR Kits (Catalog Numbers XNAT, XNATR, XNAT2, XNAT2R, XNATG, and XNATRG) have been developed for use as a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA in a 96 well format from mouse tails, hair, buccal swabs, saliva, and other animal tissues. The kits provide an integrated DNA extraction and amplification system, eliminating the need for long enzymatic digestions and homogenization steps that are not amenable to automation.

The kits contain Extract-N-Amp PCR Reaction Mix, which is an optimized reagent that includes a 2× reaction mixture of buffer, salts, dNTPs, and Taq polymerase. The reaction mix uses Sigma's antibody mediated hot start polymerase, JumpStart™ Taq polymerase, for highly specific amplification of genomic DNA directly from DNA extracts. The XNAT and XNATR kits include REDExtract-N-Amp™ PCR Reaction Mix that also contains an inert dye for convenient direct loading of the PCR reactions onto an agarose gel; whereas, the XNAT2 and XNAT2R kits are compatible with TaqMan® probes and other fluorescent-labelled probe chemistries since they do not contain dyes. Finally, XNATG and XNATRG kits contain SYBR® Green, which allows for qPCR via the fluorescent signal afforded by the binding of SYBR Green to double stranded DNA.

This automated method was created and validated for use on the Eppendorf® epMotion® Automated Pipetting System. The procedure provides a walk-away protocol for all aspects of the Extract-N-Amp Tissue PCR kit.

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

1. The Extraction and Tissue Preparation Solution is added to the tissue samples and incubated at room temperature for 10 minutes
2. The extracts are incubated for 15 minutes at 85 °C.
3. A Neutralization Solution is added to the extract. (Neutralized 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 50 minutes the Eppendorf epMotion 5075 can complete the extraction and PCR setup for 96 tissue samples.

Sigma Extract-N-Amp Tissue Kit Components

Reagents Provided	Catalog Number	Extract-N-Amp Tissue (XNAT2 and XNAT2R)		REDExtract-N-Amp Tissue (XNAT and XNATR)		Extract-N-Amp SYBR Green PCR ReadyMix (XNATG and XNATRG)	
		100 rxns	1,000 rxns	100 rxns	1,000 rxns	100 rxns	1,000 rxns
Extraction Solution	E7526	24 ml	240 ml	24 ml	240 ml	24 ml	240 ml
Tissue Preparation Solution	T3073	3ml	30 ml	3ml	30 ml	3ml	30 ml
Neutralization Solution B	N3910	24 ml	240 ml	24 ml	240 ml	24 ml	240 ml
Extract-N-Amp PCR Reaction Mix	E3004	1.2 ml	12 ml	NA	NA	NA	NA
REDExtract-N-Amp PCR Reaction Mix	R4775	NA	NA	1.2 ml	12 ml	NA	NA
Extract-N-Amp SYBR Green PCR ReadyMix	S4320	NA	NA	NA	NA	1.2 ml	12 ml

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 frost-free freezer.

User Supplied Materials

Eppendorf epMotion 5075 equipped as follows:
Thermo Module
Gripper
Dispensing Tools TM300-8 and TM50-8
Eppendorf epMotion Reservoir-Holder for 30 ml/100 ml
Thermoadaptor for 96 Deep Well Plates
Thermoadaptor for 96 PCR Plates

Eppendorf Consumables:
Reagent Reservoirs: 30 ml and 100 ml
epTIPS Motion Filtertips 50 µl & 300 µl
Eppendorf 1 ml-Deep Well Plate

Other User Supplied Materials:
96 Well PCR Polyproylene Plate (Stratagene Catalog Number 410088 or equivalent)
Animal Tissues
Small dissecting scissors
Forceps (small to medium in size)
Primers for genes of interest
Thermal Cycler for PCR

Reagent Preparation

Extraction and Tissue Preparation Solution Mixture

Pre-mix the Extraction and Tissue Preparation Solutions at a ratio of 4:1 (e.g., combine 12 ml of E7526 and 3 ml of T3073, and mix until homogeneous). This mixture can be stored up to 2 hours prior to use. To process a single plate of 96 samples, add 15 ml of the mixture to Reagent Position 1 of the Tubs-Reagent Reservoir (Deck Position- B1).

Neutralization Solution

To process a single plate of 96 samples, add 15 ml of Neutralization Solution to Reagent Position 2 of the Tubs-Reagent Reservoir (Deck Position- B1).

PCR Master Mix

To prepare the PCR Master Mix, add water and primers (forward and reverse) to the appropriate Extract-N-Amp Tissue Reaction Mix (E3004, R4775, or S4320) as described in the table, and place in Reagent Position 3 of the Tubs-Reagent Reservoir 30-100ml (Deck Position - B1):

Stock	Water	PCR Reaction Mix (E3004, R4775, or S4320)	Forward Primer (100 µM)	Reverse Primer (100 µM)
PCR Master Mix (2.4 ml)	0.9 ml	1.5 ml	10 µl	10 µl

epMotion 5075 VAC Worktable Setup

Tables 1 and 2, and Figure 1 describe the epMotion 5075 work deck layout for the automated Extract-N-Amp Tissue protocol. Arrange equipment and reagents on the work deck as describe.

Table 1.

Equipment and Reagent positioning prior to starting the run

Position: **Labware:**	**T0** Gripper	**T1** Dispensing Tool TM300-8	**T2** Dispensing Tool TM50-8	**T3** Empty	**T4** Empty
Position: **Labware:**		**A2** epTIPS Motion 300 µl Filter	**A3** epTIPS Motion 300 µl Filter	**A4** epTIPS Motion 50 µl Filter	
Position: **Labware:**	**B0** Empty	**B1** Tubs-Reagent Reservoir 30–100 ml (See Table 2)	**B2** Tissue Samples in 96 DWP	**B3** epTIPS Motion 50 µl Filter	**Vacuum** Empty
Position: **Labware:**	**WASTE**	**C1** Empty	**Temp2**** Thermal Adaptor 96 Deep Well Plate	**C3** Thermplate/AB PCR 330 (96 well PCR Plate)	**C4** Empty

**Note: Thermal module placement is instrument dependent and can be located at other deck positions.

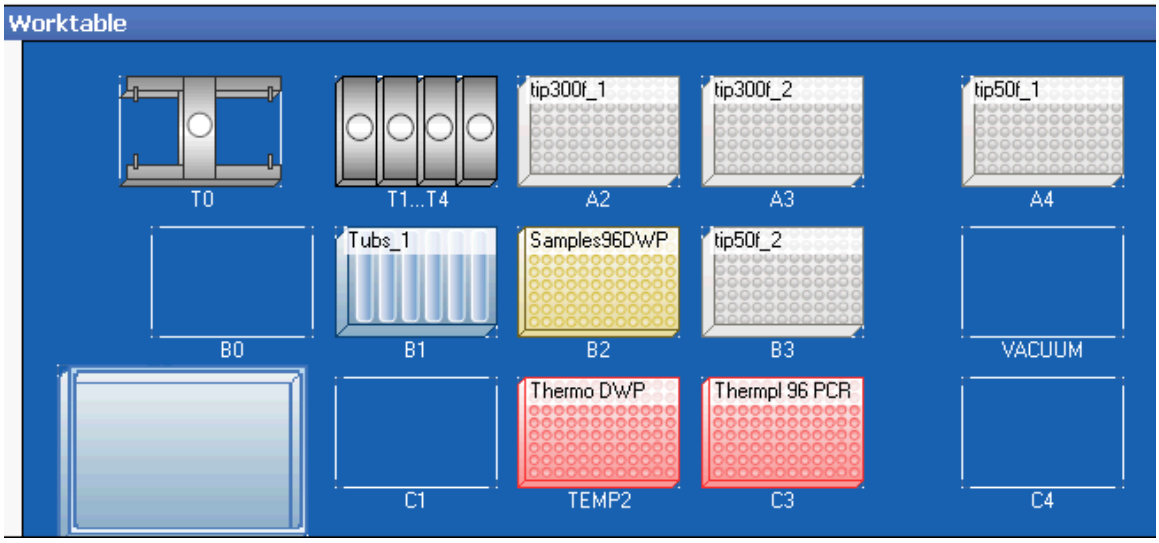
Table 2.
Placement of Reagent Reservoirs at Deck Position B1

Tubs-Reagent Reservoir 30–100 ml

Reagent Position	Reagent Reservoir Volume	Reagent name
1	**30 ml**	**Extraction –Tissue Preparation Solution**
2	**30 ml**	**Neutralization Solution**
3	**30 ml**	**PCR Master Mix**
4	NA	Empty
5	NA	Empty
6	NA	Empty
7	NA	Empty

Reagent volume in each reservoir will depend on the number of samples in each run. Please use extract reagent to account for priming of the instrument.

Figure 1.
Screenshot from the epMotion Editor showing the setup of the epMotion 5075 Vac worktable layout for the automated Extract-N-Amp Tissue PCR Kit protocol.



Tissue Preparation

For Fresh or Frozen Mouse Tails

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

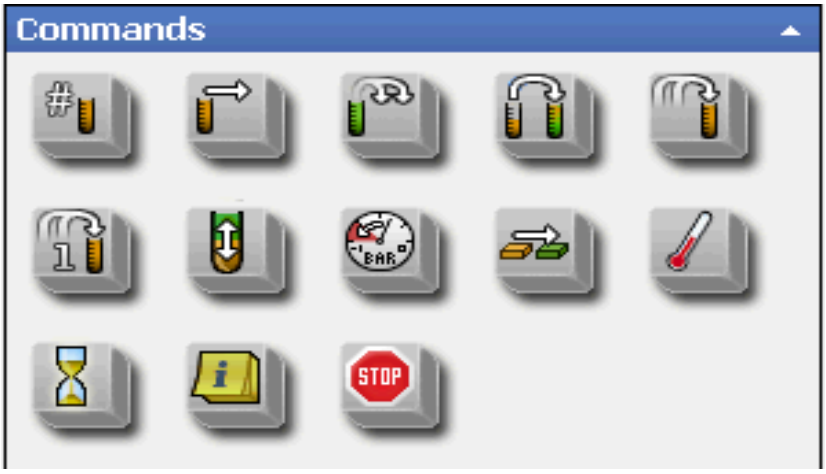
Other Animal Tissues

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 the bottom of each well. Continue with the protocol or chill the plate at 2–8 °C until needed.

epMotion 5075 Procedural Setup

1. In programming the **Procedure,** commands such as *Sample Transfer* or *Reagent Transfer* on the epMotion are click and drag functions.

Figure 2.
Screenshot of the click and drag command functions

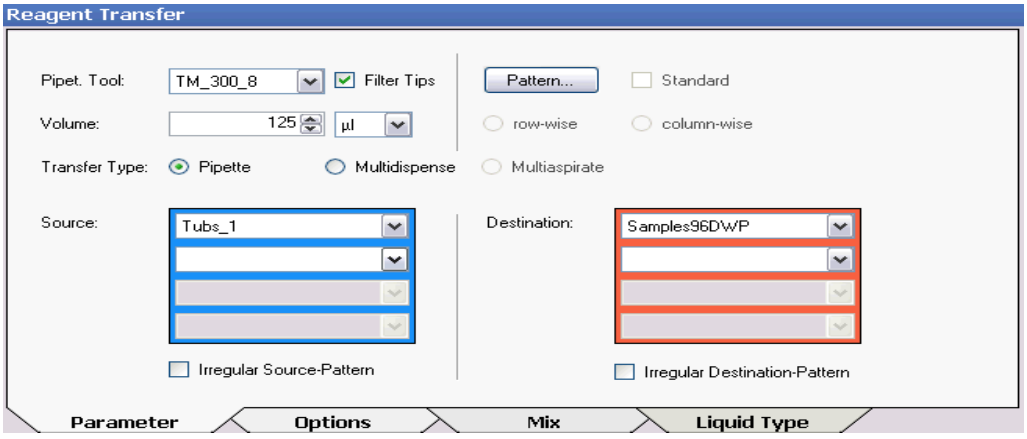


Key for the Command Functions:

# Samples	Sample Transfer	Reagent Transfer	Dilute	Pool
Pool 1 Destination	Mix	Vacuum	Transport	Temperature
Wait	Comment	Stop		

2. The **Reagent Transfer** command function launches a setup screen. The set up screen has several tabs across the bottom labeled **Parameter**, **Options**, **Mix**, and **Liquid Type**. The **Parameter** tab allows the user to choose Pipet Tool, Filter Tip, Volume, Transfer Type, Source, and Destination options. One can further define the settings for aspiration, dispensing, tip changes, mixing, pipette dispensing speed, and reagent liquid types by clicking the remaining tabs (**Options**, **Mix**, and **Liquid Type**) and manipulating the available options.

Figure 3.
Screenshot of Reagent Transfer settings



3. Procedural Commands for the Extract-N-Amp Tissue protocol are described. For further operational details please refer to the epMotion 5075 manual.

Step	Command	Key	Command Instruction
1	Temperature		TEMP2 on 85 °C
2	Number of Samples		Variable max: 96
3	Reagent Transfer		TM–300-8 125 µl - pipette Tubs 1 to Samples96DWP
4	Wait		10 min 00 sec
5	Transport		Samples96DWP to TEMP2
6	Wait		15 min 00 sec
7	Transport		Samples96DWP to B2
8	Reagent Transfer		TM–300-8 100 µl - pipette Tubs 1 to Samples96DWP
9	Reagent Transfer		TM–50-8 16.0 µl - pipette Multidisp Tubs 1 to Thermpl 96
10	Sample Transfer		TM–50-8 4.0 µl - pipette Samples96DWP to Thermpl 96
11	Temperature		TEMP2 Off
12	End of Method		(Procedure finished)

Automated Method Description - Extract-N-Amp Tissue Overview

1. Prepare samples and reagents as described in the “Tissue Preparation” and “Reagent Preparation” sections.
2. Prepare epMotion work deck with Extract-N-Amp Tissue reagents and samples as described in the “epMotion 5075 VAC Worktable Setup” section.
3. Define the number of samples and start the run. The number of samples must be defined prior to initiating the run. If the number of samples is not defined, a window will open allowing sample number entry.
4. The epMotion’s robotic carrier will begin to monitor for labware and reagent levels via an optical sensor.
5. The temperature module (TEMP2) will begin to preheat to 85 °C.
6. The robotic carrier will pick up Dispensing Tool TM300-8, which then engages an 8 set of 300 µl filter tips. 125 µl of the Extraction and Tissue Preparation Solution from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 1) is aspirated and dispensed to the tissue samples (Deck Position B2).
7. Samples are then incubated at ambient temperature for 10 minutes.
8. Next, the robotic carrier picks up the Gripper and moves the tissue samples (96 DWP) onto the 96 DWP Themoadaptor located at the TEMP2 position.
9. The samples are incubated for 15 minutes at 85 °C. Upon completion of incubation, the Gripper moves the samples back to Position B2.
10. The robotic carrier picks up Dispensing Tool TM300-8, which then engages an 8 set of 300 µl filter tips. 100 µl of Neutralization Solution from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 2) is added and mixed with the sample extracts that are now in Deck Position B2.
11. The robotic carrier picks up Dispensing Tool TM50-8, which then engages an 8 set of 50 µl filter tips. 16 µl of the PCR Master Mix from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 3) is aspirated and then multi-dispensed in the PCR plate located in the 96 PCR Plate Thermoadaptor. (Deck Position C3).
12. The robotic carrier with Dispensing Tool TM50-8, then engages an 8 set of 50 µl filter tips. 4 µl of the sample (tissue extract) from 96 DWP (Deck Position B2) is aspirated, dispensed, and mixed in the PCR plate with the PCR Master Mix (Deck Position C3).
13. Step 12 is repeated eleven times with a new set of eight tips until all 96 samples are transferred to the PCR plate.
14. The samples are now ready for PCR amplification.

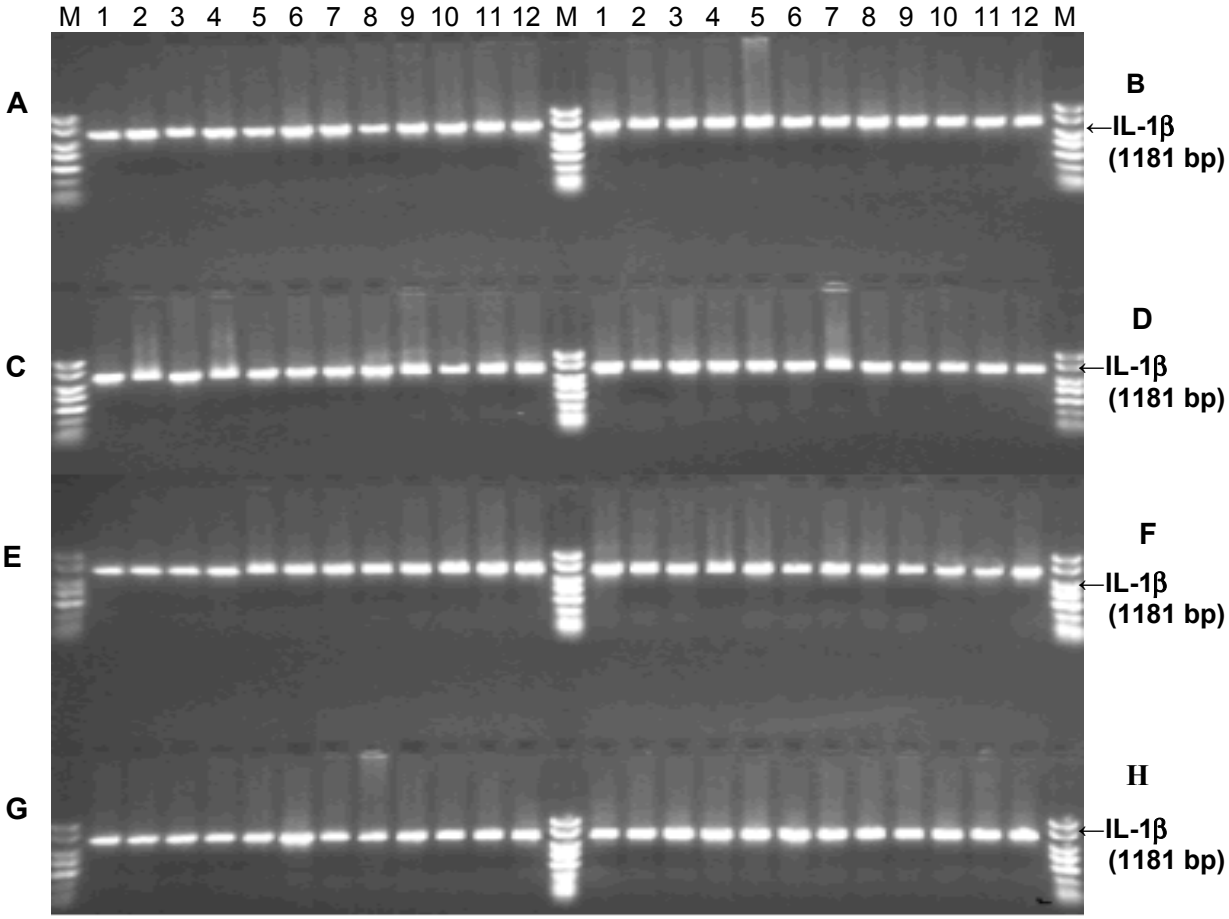
Recommended Parameters for PCR Amplification

Step	Temperature	Time	Cycles
Intial Denaturation	94–96 °C	3 minutes	1
Denaturation	94–96 °C	0.5–1 minute	
Annealing	45–68 °C	0.5–1 minute	30–40
Extension	72 °C	1–2 minutes (~kb/min)	
Final Extension	72 °C	7 minutes	1
Hold	4 °C	Indefinitely	

Performance Characteristics

The automated method was created and validated for use on the Eppendorf epMotion Automated Pipetting System. This procedure provides a walk-away protocol for all aspects of the Extract-N-Amp Tissue PCR Kit. Figure 4 shows an agarose gel of amplicons (1,181 bp IL-1β gene) generated from processing 96 different mouse-tails using the Eppendorf epMotion workstation according to the protocol outlined in this document.

Figure 4.
Agarose gel analysis of PCR from 96 Mouse Tail samples



DNA was extracted from 96 mouse tails (0.3–0.4 cm) using the automated Extract-N-Amp Tissue procedure on the Eppendorf epMotion workstation. Amplification of 1181 bp of the IL-1β gene was accomplished by combining 4 μl of DNA extract and 16 μl of 2× PCR Reaction Mix. 6 μl of resulting amplicon was resolved on a 1% agarose gel.

Troubleshooting Guide

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 the liquid handler. Adjust the aspiration or dispense of the system.
	PCR reaction is inhibited due to contaminants in the tissue extract.	Use less extract or dilute the extract with 50:50 mix of the Extraction and Neutralization Solutions and repeat the PCR
	PCR reaction is inhibited due to the presence of precipitate that may form in the tissue extract.	Centrifuge the plate containing the tissue extracts before adding the extracts to PCR amplification
	The mixing of the Neutralization Solution with tissue DNA extract is not sufficient. Alternatively, undigested tissue clogged the pipette tip.	Increase the aspiration and dispense speed and/or cycle times in the mixing steps. Dispensing can be adjusted in the epMotion parameters.
	Genomic DNA is sheared during reagent mix.	Decrease the aspiration and dispense speed and/or cycle times in the mixing steps. Dispensing can be adjusted in the epMotion parameters.
	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 PCR Kit
Negative Control shows a PCR product or “false Positive” results are present.	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 the extraction are contaminated

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