

Product Information

Automated Protocol for Extract-N-Amp™ Blood PCR Kits Using the Eppendorf® epMotion® 5075 VAC Automated Pipetting System

Description

The Extract-N-Amp™ Blood PCR Kits (Catalog Numbers XNAB, XNABE, XNABR, XNABRE, XNAB2, XNAB2E, XNAB2R, and XNAB2RE) have been developed for use as a high-throughput system for the rapid extraction and subsequent amplification of genomic DNA from whole blood, whole blood dried on a blood card, and cultured mammalian cells in a 96 well format. 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 Blood PCR ReadyMix™, 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. This ReadyMix is compatible with TaqMan® probes and other fluorescent-labelled probe chemistries. There is a second formulation of the ReadyMix, REExtract-N-Amp™ Blood PCR ReadyMix, that also contains an inert dye for convenient direct loading of the PCR reactions onto an agarose gel.

This 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 Blood PCR kit.

The extraction and amplification of genomic DNA from blood is accomplished in 3 easy steps:

1. 10 µl of Lysis Solution is added to 5 µl of whole blood and incubated at room temperature for 5 minutes.
2. 90 µl of Neutralization Solution is added to the extract. (Neutralized extracts can be stored at 4 °C for at least 6 months)
3. PCR reactions are set up using 2 µl of the extracts.

In just 35 minutes the Eppendorf epMotion 5075 can complete the extraction and PCR setup for 96 whole blood samples.

Sigma Extract-N-Amp Blood Kit Components

Reagents Provided	Catalog Number	Extract-N-Amp Blood (XNABR and XNAB2R)	Extract-N-Amp Blood (XNABRE and XNAB2RE)
		1,000 preps 1,000 rxns	1,000 preps 5,000 rxns
Lysis Solution for Blood	L3289	25 ml	25 ml
Neutralization Solution for Blood	N9784	250 ml	250 ml
Extract-N-Amp Blood PCR Ready Mix or REExtract-N-Amp Blood PCR ReadyMix	P8115 (XNAB2R & XNAB2RE) or P8240 (XNABR & XNABRE)	12 ml	5 × 12 ml

Storage

The Extract-N-Amp Blood PCR Kits can be stored at 2–8 °C for up to 3 weeks. For long-term storage, store at –20 °C in a frost-free freezer.

User Supplied Materials

Eppendorf epMotion 5075 equipped as follows:

Dispensing Tools TM300-8 and TM50-8
Eppendorf epMotion Reservoir-Holder for 30ml/100ml
Thermoadaptor for 96 PCR Plates

Eppendorf Consumables

Reagent Reservoirs: 30 ml and 100 ml
EpTIPS Motion Filtertips 50 µl & 300 µl

Other User Supplied Materials

96 Well PCR Polypropylene Plate (Stratagene Catalog Number 410088 or equivalent)
Whole blood samples
Primers for genes of interest
Thermal Cycler for PCR

Reagent Preparation

Lysis Solution

To process a single plate of 96 samples, add 5 ml of Lysis Solution to a 30 ml reagent reservoir in Position 1 of the Tubs-Reagent Reservoir (Deck Position-B1, see Figure 1).

Neutralization Solution for Blood

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, see Figure 1).

PCR Master Mix

To prepare the PCR Master Mix, add water and primers (forward and reverse) to the appropriate Extract-N-Amp Blood PCR ReadyMix (P8115 or P8240) as described in the table, and place in Reagent Position 3 of the Tubs-Reagent Reservoir (Deck Position - B1, see Figure 1):

Stock	Water	PCR Reaction Mix (E3004 or R4775)	Forward Primer (100 μ M)	Reverse Primer (100 μ M)
PCR Master Mix (2.4 ml)	0.9 ml	1.5 ml	10 μ l	10 μ l

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 Blood protocol. Arrange equipment and reagents on the work deck as described.

Table 1.

Equipment and Reagent positioning prior to starting the run.

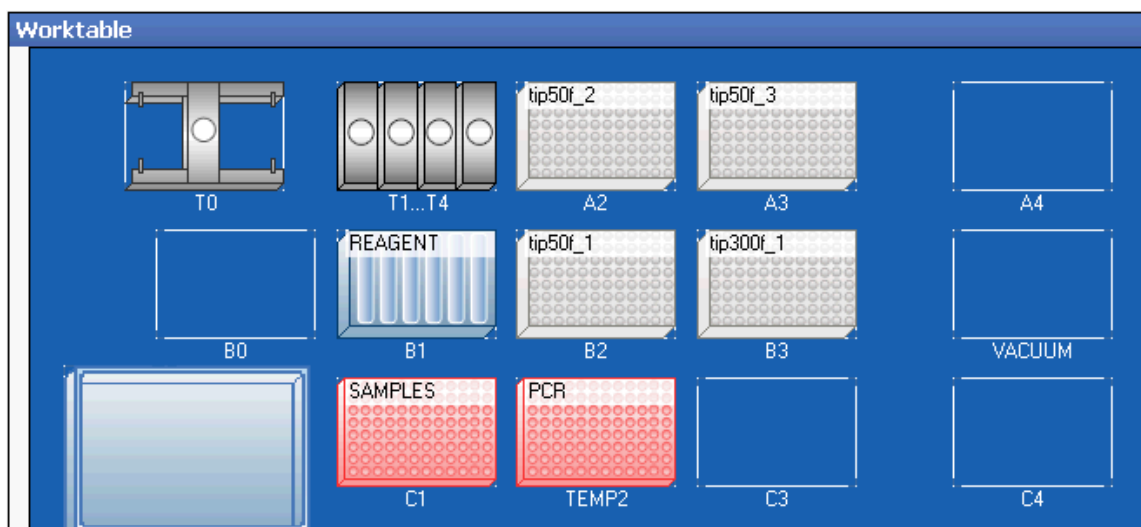
Position: Labware:	T0 Gripper	T1 Dispensing Tool TM50-8	T2 Dispensing Tool TM300-8	T3 Empty	T4 Empty
Position: Labware:		A2 EpTIPS Motion 50 μ l Filter	A3 EpTIPS Motion 50 μ l Filter	A4 Empty	
Position: Labware:	B0 Empty	B1 Tubs-Reagent Reservoir 30–100 ml (See Table 2)	B2 EpTIPS Motion 50 μ l Filter	B3 EpTIPS Motion 300 μ l Filter	Vacuum Empty
Position: Labware:	WASTE	C1 Samples (96 well plate) in Thermplate/AB PCR 330	C2 Empty 96 well PCR plate in Thermplate/AB PCR 330	C3 Empty	C4 Empty

Table 2.
Placement of Reagent Reservoirs at Deck Position B1

Tubs-Reagent Reservoir 30–100ml		
Position size	Maximum Reagent Reservoir Volume	Reagent name
1	30 ml	Lysis Solution for Blood
2	30 ml	Neutralization Solution for Blood
3	30 ml	PCR Master Mix
4		Empty
5		Empty
6		Empty
7		Empty

Reagent volume in each reservoir will depend on the number of samples in each run. Please use extra 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 Blood PCR Kit protocol.

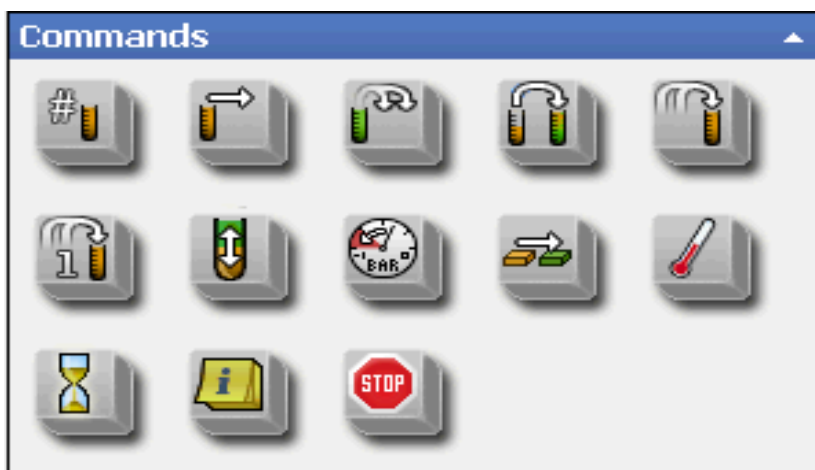


epMotion 5075 Procedural Setup

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		

- 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.

Note: Appropriate mixing is required for optimal results with the Blood Extract-N-Amp protocol. See recommended Mix settings.

Figure 3.
Screenshot of Reagent Transfer settings

Reagent Transfer

Pipet Tool: TM_300_8 Filter Tips Standard

Volume: 125 µl row-wise column-wise








Transfer Type: Pipette Multidispense Multiaspirate

Source: Tubs_1 Destination: Samples96DWP

Irregular Source-Pattern Irregular Destination-Pattern

Parameter Options Mix Liquid Type

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

Step	Command	Key	Command Instruction						
1	Number of Samples		Variable max: 96						
2	Reagent Transfer		<p>TM-50-8 10 μl - pipette REAGENT (1) to SAMPLES Mix</p> <table border="1"> <tr> <td>No. Cycles</td> <td>8</td> </tr> <tr> <td>Speed:</td> <td>15</td> </tr> <tr> <td>Volume:</td> <td>6 μl</td> </tr> </table>	No. Cycles	8	Speed:	15	Volume:	6 μl
No. Cycles	8								
Speed:	15								
Volume:	6 μl								
3	Wait		5 min 00sec						
4	Reagent Transfer		<p>TM-300-8 90 μl - pipette REAGENT (2) to SAMPLES Mix</p> <table border="1"> <tr> <td>No. Cycles</td> <td>8</td> </tr> <tr> <td>Speed:</td> <td>15</td> </tr> <tr> <td>Volume:</td> <td>50 μl</td> </tr> </table>	No. Cycles	8	Speed:	15	Volume:	50 μl
No. Cycles	8								
Speed:	15								
Volume:	50 μl								
5	Reagent Transfer		TM-50-8 18 μ l - pipette (Multidisp) REAGENT to PCR						
6	Sample Transfer		<p>TM-50-8 2 μl - pipette SAMPLES to PCR Mix</p> <table border="1"> <tr> <td>No. Cycles</td> <td>4</td> </tr> <tr> <td>Speed:</td> <td>10</td> </tr> <tr> <td>Volume:</td> <td>10 μl</td> </tr> </table> <p>After mixing, aspirate from bottom</p>	No. Cycles	4	Speed:	10	Volume:	10 μl
No. Cycles	4								
Speed:	10								
Volume:	10 μl								
7	End of Method		(Procedure finished)						

Automated Method Description

Extract-N-Amp Blood Overview

1. Prepare reagents as described in the “Reagent Preparation” section.
2. Prepare epMotion work deck with Extract-N-Amp Blood reagents as described in the “epMotion 5075 VAC Worktable Setup” section. Place a 96 well plate containing 2 μ l of whole blood per well in Deck Position C1.
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 robotic carrier will pick up Dispensing Tool TM50-8, which then engages an 8 set of 50 μ l filter tips. 10 μ l of the Lysis Solution from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 1) is aspirated, dispensed to the blood samples in Deck Position C1, and mixed.
6. Samples are then incubated at ambient temperature for 5 minutes.
7. The robotic carrier will pick up Dispensing Tool TM300-8, which then engages an 8 set of 300 μ l filter tips. 90 μ l of Neutralization Solution from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 2) is added and mixed with the blood sample extracts in Deck Position C1.
8. Next, the robotic carrier picks up Dispensing Tool TM50-8, which then engages an 8 set of 50 μ l filter tips. 18 μ l of the PCR Master Mix from Tub-Reagent Reservoir (Deck Position B1-Reagent Position 3) is aspirated and multi-dispensed into the PCR plate located in Deck Position C2 [Temp2].
9. The robotic carrier with Dispensing Tool TM50-8, then engages an 8 set of 50 μ l filter tips. 2 μ l of the blood extract from Deck Position C1 is aspirated, dispensed, and mixed in the PCR plate with the PCR Master Mix (Deck Position C2 [Temp2]).
10. Step 9 is repeated with a new set of eight tips until all samples are transferred to the PCR plate.
11. The samples are now ready for PCR amplification.

Recommended Parameters for PCR Amplification

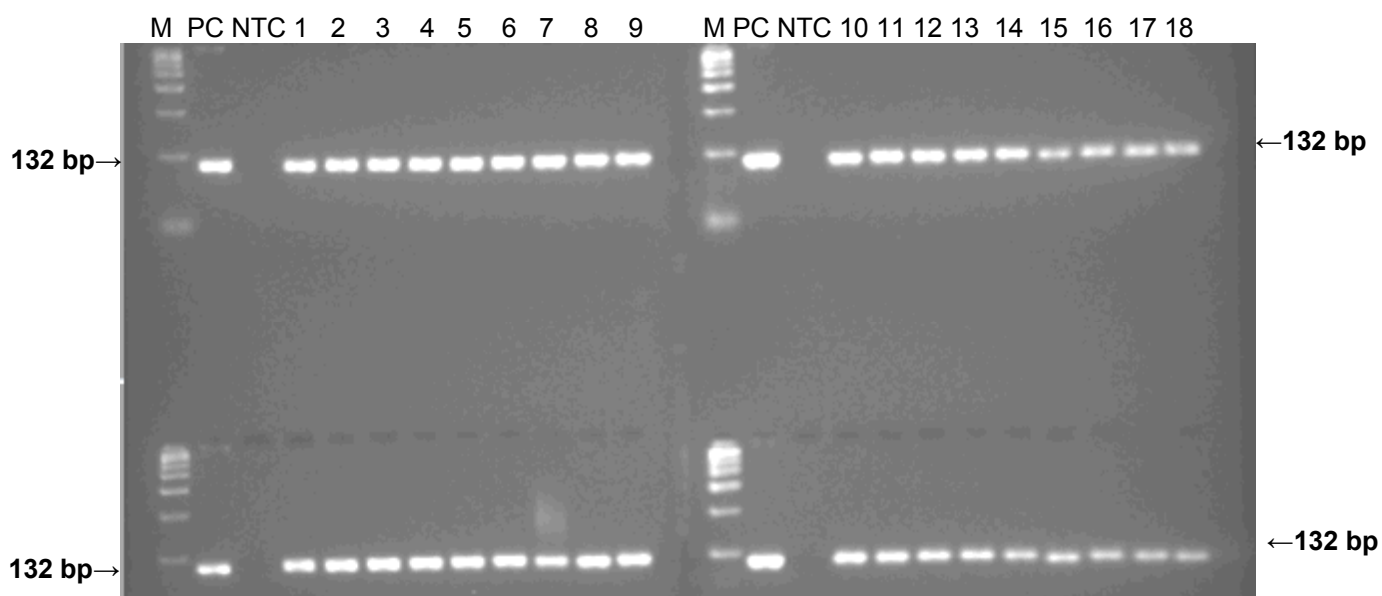
Step	Temperature	Time	Cycles
Initial Denaturation	94–96 °C	3 minutes	1
Denaturation	94–96 °C	0.5–1 minute	
Annealing	45–68 °C	0.5–1 minute	30–40
Extension	72 °C	1 minute (1 minute/kb)	
Final Extension	72 °C	7 minutes	1
Hold	4 °C	Indefinitely	

Performance Characteristics

The automated method described 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 Blood PCR kit. Figure 4 shows an agarose gel of amplicons (132 bp STB39 gene) generated from processing 36 different whole blood samples using the Eppendorf epMotion workstation according to the protocol outlined in this document.

Figure 4.

Agarose gel analysis of PCR from 36 blood samples



DNA was extracted from 36 whole blood samples using the REExtract-N-Amp PCR procedure on the Eppendorf epMotion. Amplification of 132 bp of the STB39 gene was accomplished by combining 2 μ l of the extracted template and 18 μ l of 2 \times PCR Reaction Mix. 6 μ l of each amplicon was then resolved on a 4% agarose gel. The positive control was human genomic DNA.

Troubleshooting Guide

Problem	Cause	Solution
Little or no PCR product is detected.	PCR reaction is inhibited due to contaminants in the blood extract.	Use less extract or dilute the extract with water, include a DNA control, and/or spike a known amount of template (100–500 copies) into the PCR mixture along with the blood extract to act as a control for inhibition; lack of PCR product in the spiked sample indicates the presence of PCR inhibitors.
	A PCR component is missing or degraded.	Run a positive control to ensure components are functioning. A checklist is also recommended when assembling reactions.
	Too few cycles are performed.	Increase the number of cycles (5–10 additional cycles at a time).
	The annealing temperature is too high.	Decrease the annealing temperature in 2–4 °C increments until the optimal temperature is empirically determined.
	The primers are not 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 extension time is too short.	Increase the extension time in 1-minute increments especially for long templates. In general, use 1-minute of extension time for every 1 kb of product length (e.g., use a 5-minute extension for a 5 kb amplicon).
	The target template is complex.	In most cases, inherently complex targets are due to unusually high GC content and/or secondary structure. Betaine has been reported to help amplification of high GC content targets when supplemented into PCR reactions at a concentration of 1.0–1.7 M.
Multiple products are seen.	JumpStart Taq antibody is not working correctly.	Do not use DMSO or formamide with REExtract-N-Amp PCR ReadyMix. It can interfere with the enzyme-antibody complex. Addition of solvents and salt, or extremes in pH or reaction conditions may reduce the affinity of the JumpStart Taq antibody for the Taq Polymerase and thereby, compromise its effectiveness.
	The annealing temperature is too low.	Increase the annealing temperature in 2–4 °C increments until the optimal temperature is empirically determined.
Negative Control shows a PCR product or “false positive” results are present.	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 the extraction or PCR are contaminated with template from a previous reaction.

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Patent Pending

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