

Applications

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Automated Protocol for Sigma's Extract-N-Amp™ Blood PCR Kits using the Eppendorf epMotion® 5075 VAC

Steve Michalik, Sigma-Aldrich Biotech, St. Louis, MO, USA

Renate Fröndt, Eppendorf AG, Hamburg, Germany

Abstract

The Extract-N-Amp Blood PCR Kits 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. Using the Eppendorf epMotion 5075 VAC, the extraction and PCR setup for 96 whole blood samples can be completed in just 35 minutes.

Introduction

The Extract-N-Amp Blood PCR Kits contain Extract-N-Amp Blood PCR ReadyMix, which is an optimized reagent that includes a 2X 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. Lysis Solution is added to whole blood and incubated at room temperature for 5 minutes. A Neutralization Solution is added to the extract. The neutralized extracts can be stored at 4 °C for at least 6 months. 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.

Materials and Methods

Sigma Extract-N-Amp Blood Kit (Product codes XNAB, XNABE, XNABR, XNABRE, XNAB2, XNAB2E, XNAB2R and XNAB2RE) Components

Lysis Solution for Blood
 Neutralization Solution for Blood
 Extract-N-Amp Blood PCRReady Mix
 REExtract-N-Amp Blood PCR ReadyMix

Eppendorf epMotion 5075 VAC equipped as follows:

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

Eppendorf Consumables:

Reagent Reservoirs: 30 mL and 100 mL
 epT.I.P.S Motion Filtertips 50 µL and 300 µL

Other User-supplied Materials:

96 Well PCR Polypropylene Plate
 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 (worktable 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 (worktable 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 below, and place in reagent position 3 of the Tubs-Reagent Reservoir (worktable position-B1, see figure 1):

Mastermix (2.4 ml)	
Water	0.9 mL
PCR Reaction Mix (E 3004 or R 4775)	1.5 mL
Forward Primer (100 µM)	10 µL
Reverse Primer (100 µM)	10 µL

Table 1: PCR Master Mix

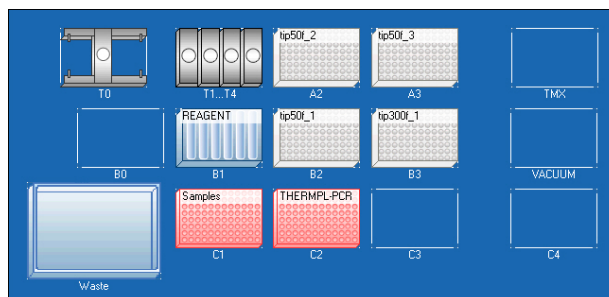


Figure 1: Screenshot from epMotion Editor showing the setup of the epMotion 5075 VAC worktable layout for the automated Extract-N-Amp Blood PCR Kit protocol

Automated Method Overview

The prepared samples and reagents are set up in the worktable layout as described.
 Upon executing the run, if the number of samples is defined as variable, then a window opens to allow entry of the numbers of samples to be processed.
 The extraction will start with 10 µL of the Lysis Solution from Tub-Reagent Reservoir to the samples.

Worktable Position	Labware
A2	50 µL epTips, Filtertips
A3	50 µL epTips, Filtertips
B1	Reservoir Rack with 3 Reagent Reservoirs 30 mL
B2	50 µL epTips, Filtertips
B3	300 µL epTips, Filtertips
C1	Thermoblock with PCR Plate with samples
C2 or TEMP2	Thermoblock with PCR Plate, empty

Table 2: epMotion worktable allocation for Extract-N-Amp Blood PCR Kit

Results and Summary

The automated method described above was created and validated for use on the *epMotion* automated pipetting system. This procedure provides a walk-away protocol for all aspects of the Extract-N-Amp Blood PCR kit. Figure 2 shows an agarose gel of amplicons (132 bp STB39 gene) generated from processing 36 different whole blood samples using *epMotion* workstation according to the protocol outlined in this document.

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

Table 3: Recommended Parameters for PCR Amplification

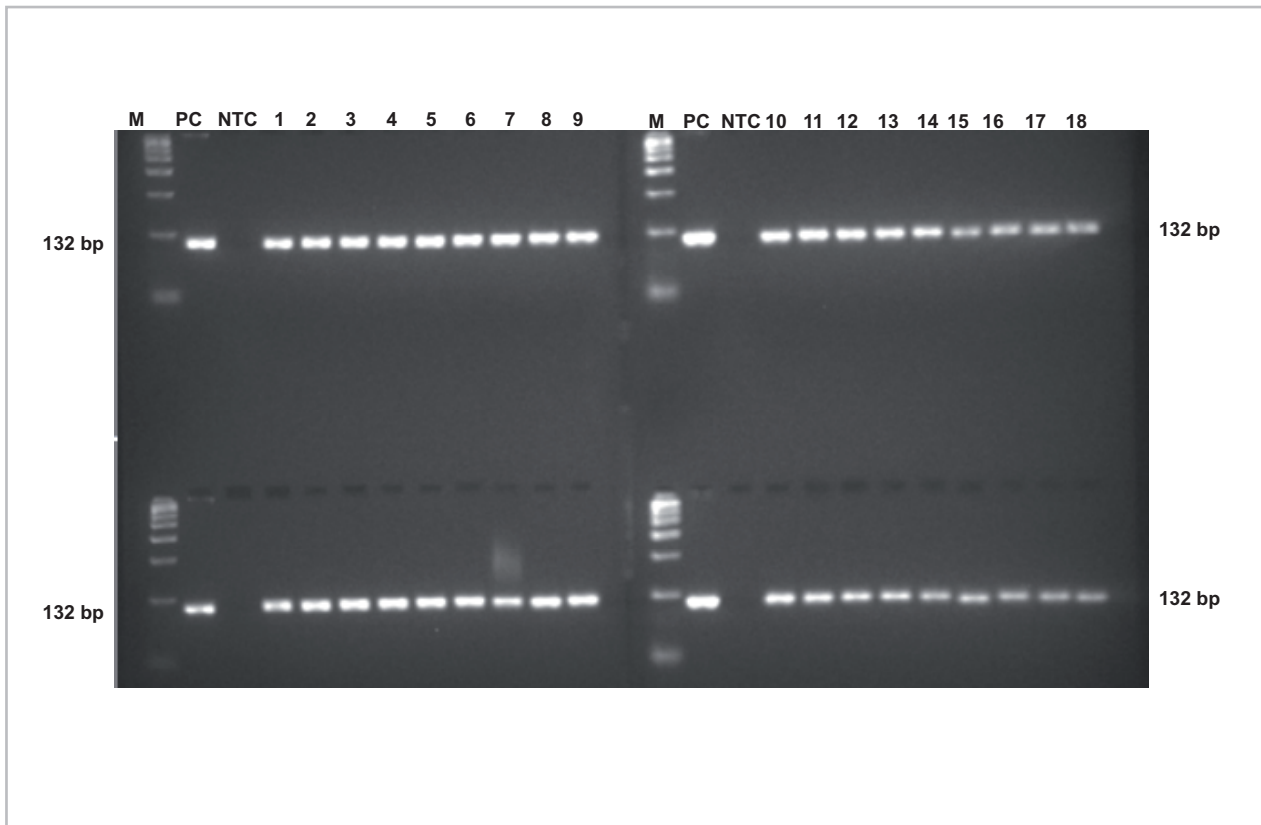


Figure 2: Agarose gel analysis of 36 blood samples.

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 2X PCR Reaction Mix. 6 μ L of each amplicon was then resolved on a 4 % agarose gel.

M: DNA ladder

PC: positive control (human genomic DNA)

NTC: negative control (non-template control).

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
epMotion® 5075 VAC, 230 V	5075 000.016	N/A
epMotion® 5075 VAC, 120 V	N/A	960020014
epMotion® 5075 LH, 230 V	5075 000.008	N/A
epMotion® 5075 LH, 120 V	N/A	960020006
Dispensing tool TM 300-8	5280 000.231	960001052
Dispensing tool TM 50-8	5280 000.215	960001044
Reservoir Rack	5075 754.002	960002148
epMotion Reservoir 30 mL	0030 126.505	960051009
epT.I.P.S® Motion 300 µL, Filter	0030 003.977	960050061
epT.I.P.S® Motion 50 µL, Filter	0030 003.950	960050029
Thermoblock for 96 PCR Plates	5075 766.000	960002083

Ordering Information Sigma

Product	Order no. international
Lysis Solution	L3289
Neutralization Solution for Blood	N9784
Extract-N-Amp™ Blood PCR Ready Mix	P8115
REDEExtract-N-Amp™ Blood PCR Ready Mix	P8240



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Eppendorf AG · 22331 Hamburg · Germany · Tel: +49 40 53801-0 · Fax: +49 40 538 01-556 · E-mail: eppendorf@eppendorf.com

Eppendorf North America, Inc. · 102 Motor Parkway · Hauppauge, N.Y. 11788-5178 · USA

Tel: +1 516 334 7500 · Toll free phone: +1 800-645-3050 · Fax: +1 516 334 7506 · E-mail: info@eppendorf.com

Application Support Europe, International: Tel: +49 1803 666 789 (Preis je nach Tarif im Ausland; 9 ct/min aus dem dt. Festnetz; Mobilfunkhöchstpreis 42 ct/min) · E-mail: support@eppendorf.com

North America: Tel: +1 800 645 3050 · E-mail: techserv@eppendorf.com

Asia Pacific: Tel: +60 3 8023 6869 · E-mail: support_asiapacific@eppendorf.com