

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

Product Information

Using the Sciclone ALH 3000 Workstation (Caliper Life Sciences)

Extract-N-Amp Blood Product Codes **XNABR** and **XNAB2R**

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Automation Guide

I. Description

The Extract-N-Amp Blood PCR kit has been developed for use as a high throughput system for the rapid extraction and subsequent amplification of genomic DNA from whole blood in a 96 well format. The Extract-N-Amp Blood PCR kits provide a novel extraction system that eliminates the need for any type of purification, organic extraction, centrifugation, heating, filtration, or alcohol precipitation. 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 mechanism, JumpStart™, for highly specific amplification of genomic DNA directly from the extract. There is also a second formulation of the Ready Mix, REExtract-N-Amp PCR ReadyMix that contains a 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 walk-away protocol for all aspects of the Extract-N-Amp Blood PCR kit.

Extraction and amplification of genomic DNA from whole 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. Extracts are now stable for at least 6 months if stored at 4 °C.
3. PCR reactions are set up using 2 µl of the extracts.

In just 25 minutes, the Sciclone ALH 3000 can complete extraction and PCR reaction setup of 96 whole blood samples.

II. Product Components

Reagents Provided	Product Code	Extract-N-Amp Blood XNAB2R	REExtract-N-Amp Blood XNABR
	Package Size	1000 extractions 1000 amplifications	1000 extractions 1000 amplifications
Lysis Solution for Blood	L 3289	25 ml	25 ml
Neutralization Solution for Blood	N 9784	250 ml	250 ml
Extract-N-Amp PCR Ready Mix or REExtract-N-Amp PCR Ready Mix	P 8115 (for XNAB2R) P 8240 (for XNABR)	12 ml	12 ml

III. 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.

IV. Materials to Be Supplied by the User

1. Whole blood
2. Primers for human genes of interest
3. Molecular biology grade water (Sigma-Aldrich, W 4502)
4. 96 well polypropylene, round bottom multiwell plates (Sigma-Aldrich, P 6866)
5. 96 well PCR amplification plates, with half skirt (ABgene, AB-1100)
6. Ultra clear cap strip (ABgene, AB-0866)
7. Corning plate holder (Corning, 6525)
8. Sealing film, SealPlate (Sigma-Aldrich, Z36,965-9)
9. Microcentrifuge tubes (1.5 ml, 2 ml screw cap)
10. 24-position Eppendorf IsoTherm System (Fisher, 05-405-22)
11. 12 column reagent reservoir with low profile (Innovative Microplates, S30028)
12. 96 well reservoir with low profile and pyramidal bottom (Innovative Microplates, S30018)
13. (Optional) 12 column reagent reservoir with high profile (Innovative Microplates, S30019)
14. (Optional) 96 well reservoir with high profile and pyramidal bottom (Innovative Microplates, S30014)
15. Thermal Cycler

V. Blood Collection

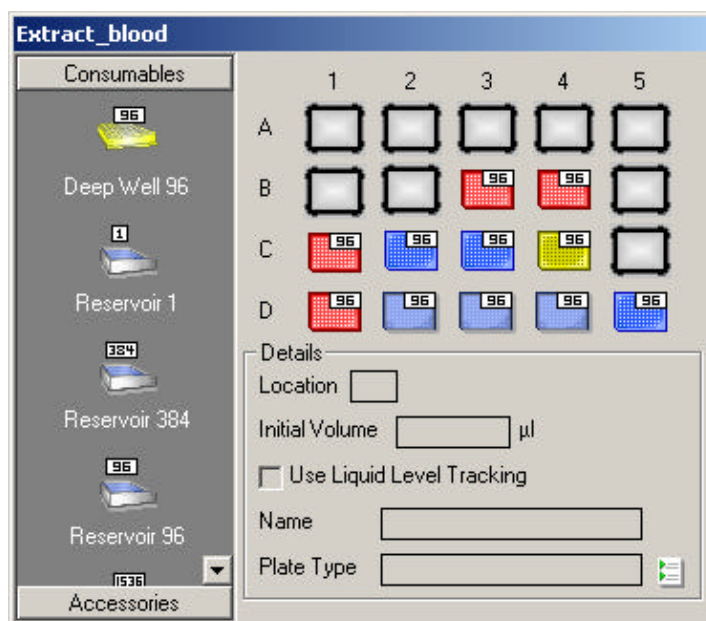
Observe standard precautions when handling blood or blood products.

1. Collect blood into tubes containing EDTA, sodium citrate, or sodium heparin. The best results may be obtained with EDTA or sodium citrate. Mix thoroughly by inverting collection tubes two to three times.
2. Chill the tubes at 2-8 °C until needed.
3. Carefully aliquot 5 µl of each blood sample into individual wells of a 96 well polypropylene multiwell plate ensuring that each sample is centered down into the bottom of each well. Seal plate with sealing film until needed.

VI. Instrument Requirements for the Sciclone ALH 3000 Workstation

Part Description	Qty	Ordering Information
Deck Mounted Shaker	1	Contact Caliper
96 channel High Volume Head	1	Contact Caliper
Z8™ Pipettor	1	Contact Caliper
Gripper	1	Contact Caliper
I/O Box	1	Contact Caliper
Deck Locator	6	Contact Caliper
Tip Box Locator	4	# 76523 (Caliper)
100 µl Disposable Tip Box	1	# 66670 (Caliper)
80 µl Barrier Tip Box	2	# 68759 (Caliper)
200 µl Disposable Tip Box	1	# 56362 (Caliper)

VII. Deck Setup



Deck Position	Equipment
B3	100 µl Tip box
B4	80 µl Tip box, Barrier Tips
C1	200 µl Tip box
C2	96 well polypropylene multiwell plate with blood samples
C3	96 well PCR amplification plate (seated into a plate holder)
C4	24 position Eppendorf IsoThem system
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 Lysis Solution
D5	Shaker

VIII. Reagent Preparation

1. *Lysis Solution*: To process a single plate of 96 samples, add 20 ml of Lysis Solution to 96 well reservoir S30018 located at position D4.
2. *Neutralization Solution*: To process a single plate of 96 samples, add 30 ml of Neutralization Solution to 96 well reservoir located at position D2. This reservoir has a maximum capacity of 80 ml, and if processing more than 5 plates of samples it may be necessary to use a larger reservoir S30014.
3. *PCR Master Mix*: The Extract -N-Amp Blood PCR ReadyMix is a 2x reaction mixture containing buffer, salts, dNTPs, and *Taq* polymerase. To prepare a PCR Master Mix add water, and forward and reverse primers to the Extract-N-Amp Blood PCR ReadyMix as described in table below.

	Water	PCR Mix	Forward Primer	Reverse Primer
Stock		P 8115	5 μ M	5 μ M
Working (3.6 ml)	0.8 ml	2 ml	0.4 ml	0.4 ml

To set up one multiwell plate of 20 μ l PCR reactions, 3.6 ml of the PCR Master Mix should be placed in the second column of reservoir S30028 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 into 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 quantitation of the blood DNA extracts. Genomic DNA solutions of 3.2 ng/ μ l, 1.6 ng/ μ l, 0.8 ng/ μ l, and 0.2 ng/ μ l were prepared and placed on column 4 of 24-position tube rack located at position C4.

IX. Automated Method Description

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

A. General Procedures:

1. Set up deck layout: place the tip boxes, plates, tube rack, and reservoirs at the appropriate positions on the deck as described in section VII.
2. Add reagents to the appropriate reservoirs as described in section VIII.
3. Run the method using Sciclone Software Version 3.2.
4. At the completion of the method, place the cap strips onto the PCR plate, vortex to mix the solution and briefly centrifuge. The PCR plate is now ready for placement into a thermal cycler.
5. Blood 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_Blood: Performs all of the steps necessary to extract DNA from 96 blood samples and setup PCR reactions.

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

Extract-N-Amp_Blood: Method Overview

Below is a summary of the *Extract-N-Amp_Blood* automated method. For complete program details download automation program at www.sigmaldrich.com/automation

1. Speed of Sciclone head movement is set at 80%.
2. 100 µl disposable tips are loaded onto the 96 channel high volume head.
3. 10 µl of Lysis Solution is aspirated from a reservoir by the 96 channel high volume head.
4. Lysis Solution is dispensed into multiwell plate containing blood samples.
5. The 96 channel high volume head is used to pipette -mix blood samples to prepare extracts. 8 cycles of mixing are performed.
6. 100 µl disposable tips are removed from the 96 channel high volume head.
7. Gripper tool is used to move the plate containing blood extracts to the shaker.
8. Shaker is activated to begin mixing plate with blood extracts.
9. A timer allows for 30 seconds of uninterrupted plate mixing.
10. Shaker is inactivated.
11. A timer commences for a 5-minute incubation at room temperature.
12. 200 µl disposable tips are loaded onto the 96 channel high volume head.
13. 90 µl of Neutralization Solution is aspirated from a reservoir by the 96 channel high volume head.
14. Neutralization Solution is dispensed into the multiwell plate with the blood extracts.
15. The 96 channel high volume head is used to pipette-mix the extracts for 6 cycles.
16. 200 µl disposable tips are removed from the 96 channel high volume head.
17. Shaker is activated to begin mixing plate with blood extracts.
18. A timer allows for 30 seconds of uninterrupted plate mixing.
19. Gripper tool is used to move plate containing blood extracts from deck position D5 to C2.
20. A command calls up and performs all steps of the *PCR_Setup* Method. See Below for explanation of this method.

PCR_Setup: Method Overview

Below is a summary of the *PCR_Setup* method. For complete program details, download automation program from www.sigmaldrich.com/automation

1. Speed of Sciclone head movement is set at 80%.
2. 80 µl barrier disposable tips are loaded onto the Z-8 dispense head.
3. PCR Master Mix is aspirated from the 12 -column reservoir using the Z-8 dispense head. The Z-8 is acting like a bulk reagent dispenser, and is aspirating enough reagent to dispense to a quarter of the plate.
4. PCR Master Mix is multi -dispensed to the PCR amplification plate using the Z-8 dispense head
5. Steps 3 and 4 are repeated 3 more times until the Z-8 has dispensed 18 µl of PCR Master Mix to all 96 wells of the PCR amplification plate.
6. 80 µl barrier tips are removed from the Z-8 dispense head.

7. 100 μ l disposable tips are loaded onto the Z-8 dispense head.
8. 2 μ l of blood extracted is aspirated from the multiwell plate used during the Extract-N-Amp Blood method.
9. Blood extract is dispensed into the PCR amplification plate.
10. 100 μ l disposable tips are removed from the Z-8 dispense head.
11. Because the Z-8 dispense head can only perform operations eight wells at a time, a loop is created to account for all samples. Steps 7-10 are repeated 10 times.
12. 100 μ l disposable tips are loaded onto the Z-8 dispense head
13. 2 μ l of control DNA samples or water are aspirated using the Z-8 dispense head from microfuge tubes.
14. Control DNA samples and water are dispensed to the last column of the PCR amplification plate using the Z-8 dispense head.
15. 100 μ l disposable tips are removed from the Z-8 dispense head.

X. 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	

XI. Method Customization

PCR setup only

Blood extracts may be subjected to additional amplifications. The *PCR_Setup* method 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. 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 touch is critical for the addition of the low volume of blood DNA extract to the reaction mixture.

PCR setup using multiple primer sets

To amplify genomic DNA from the 96 blood extracts with different primer sets, primers can be added to microfuge tubes and placed on 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.

XII. Performance Characteristics

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

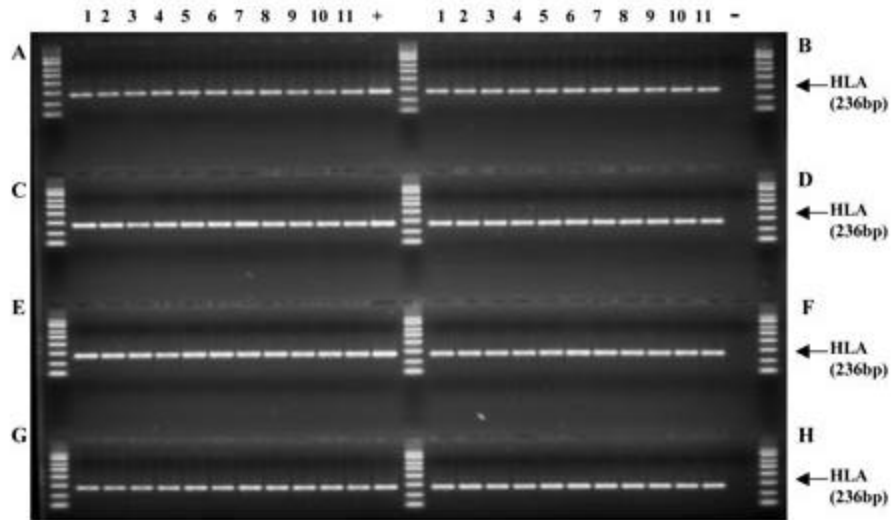


Figure 1. Agarose gel analysis of 96 PCR samples. DNA was extracted from 88 samples of human whole blood (5 μ l) from a single donor using the automated Extract-N-Amp™ Blood PCR procedure on the Sciclone ALH 3000. Amplification of 236 bp fragment of the HLA gene followed using 2 μ l of extracted template and 2 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 2% Agarose gel.

Cross-Contamination Analysis

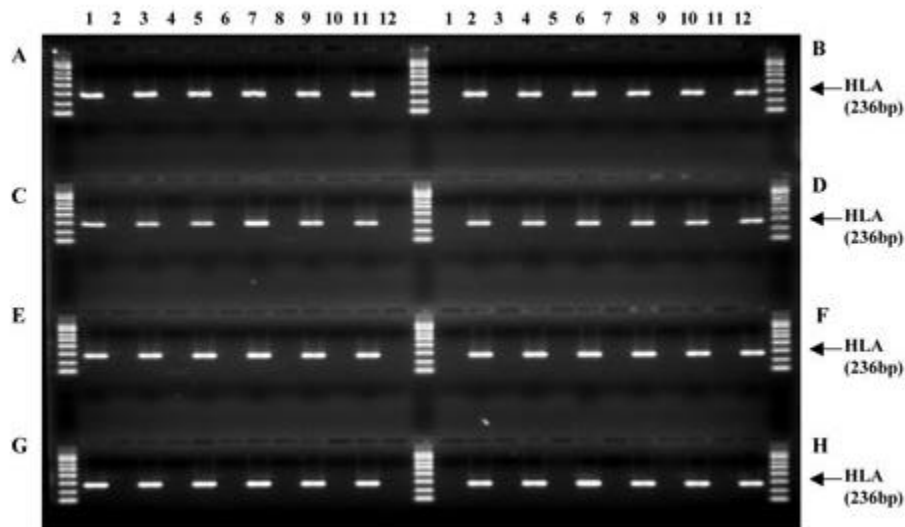


Figure 2. Cross-contamination analysis. 5 μ l samples of blood or PBS were placed in alternating wells of an extraction plate. The 96 well plate was processed using the automated Extract-N-Amp Blood PCR procedure on the Sciclone ALH 3000. All samples were then subjected to amplification and 6 μ l of the resultant products were electrophoresed on a 2% Agarose gel. No PCR products were detected in the samples containing PBS controls.

XIII. 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 blood 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 blood extract.	Use less extract or dilute the extract with water and repeat PCR.
	The extraction of blood DNA is not sufficient due to inefficient mixing by the Sciclone during lysis step.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps. Observe the color change (from dark red to dark brown) of blood sample after thorough mixing with Lysis Solution.
	Genomic DNA is sheared by mixing 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™ Blood 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.

XIV. Contact Information

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

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

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