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

Extract-N-Amp™ Blood PCR Kits

Catalog Numbers **XNAB2, XNAB2E, XNAB2R, XNAB2RE, and P8115**

TECHNICAL BULLETIN

Product Description
The Extract-N-Amp Blood PCR Kits contain all the reagents needed to rapidly extract and amplify human genomic DNA from whole blood, whole blood dried on a blood card, and cultured mammalian cells. Briefly, DNA is released by incubating the sample with the Lysis Solution at room temperature for 5 minutes for whole blood, at 55 °C for 15 minutes for blood cards, or at 75 °C for 5-10 minutes for cell monolayers. After adding the Neutralization Solution, the extract is ready for PCR.

An aliquot of the neutralized extract is then combined with the Extract-N-Amp Blood PCR ReadyMix™ and user-provided PCR primers to amplify the target DNA. The Extract-N-Amp Blood PCR ReadyMix is a 2x reaction mixture containing buffer, salts, dNTPs, and *Taq* polymerase. It also contains the JumpStart™ antibody for hot start PCR to enhance specificity. Note that the Extract-N-Amp Blood PCR ReadyMix has the same formulation as the REDExtract-N-Amp™ Blood PCR ReadyMix, except that the red dye is omitted. This allows for use with detection methods in which the dye interferes.

Reagents Provided	**Catalog Number**	**XNAB2** 100 preps, 100 PCRs	**XNAB2E** 100 preps, 500 PCRs	**XNAB2R** 1,000 preps, 1,000 PCRs	**XNAB2RE** 1,000 preps, 5,000 PCRs
Lysis Solution for Blood	L3289	2.5 ml	2.5 ml	25 ml	25 ml
Neutralization Solution for Blood	N9784	25 ml	25 ml	250 ml	250 ml
Extract-N-Amp Blood PCR ReadyMix. **This is a 2x PCR reaction mix** containing buffer, salts, dNTPs, *Taq* polymerase, and JumpStart antibody.	P8115	1.2 ml	5 x 1.2 ml	12 ml	5 x 12 ml

Reagents and equipment required, not provided
- Microcentrifuge tubes or multiwell plate for extractions (200 µL minimal volume)
- Punch and cards for dried blood
- Incubator or oven for blood cards (55 °C) or monolayer cells (75 °C)
- Tubes or plate for PCR
- Thermal cycler
- PCR primers
- Water, PCR reagent, Catalog Number W1754

Storage/Stability
The Extract-N-Amp Blood PCR Kits can be stored at 2-8 °C on a short-term basis up to 3 weeks. For long-term storage greater than 3 weeks, –20 °C is recommended. Do not store in a "frost-free" freezer.

Precautions and Disclaimer
The Extract-N-Amp Blood PCR Kits are for R&D use only, not for drug, household or other uses. The Lysis Solution is caustic. Avoid contact with skin. Wear gloves, safety glasses, and suitable protective clothing when handling this or any other reagent provided with the kit. Consult the MSDS for information regarding hazards and safe handing practices.

Procedure

All steps are carried out at room temperature unless otherwise noted.

A. DNA extraction from Whole Blood

1a. 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 inversion or rocking.
Note: For non-human sources, collect blood into tripotassium EDTA, Catalog Number E0270, at a final concentration of 5 mM to prevent coagulation.

2a. Place 20 µL of the Lysis Solution for Blood into a microcentrifuge tube or well of a multiwell plate for each extraction.

3a. Add 10 µL of blood. Mix thoroughly by vortexing or pipetting.

4a. Incubate at room temperature for 5 minutes.

5a. Add 180 µL of the Neutralization Solution for Blood. Mix thoroughly by vortexing or pipetting.

6a. Store the neutralized blood extract at 4 °C or use 2 µL immediately in PCR. Continue with step 7.
Note: DNA is stable in the extract for at least 6 months at 4 °C.

B. DNA extraction from Blood Cards

1b. Collect the blood sample onto a collection card, Catalog Number C2613. Allow to dry completely.

2b. Punch a disk (preferably 1/8 inch or 3 mm) from the blood card and place into a microcentrifuge tube. Make sure that the punch contains as much of the blood-stained area as possible.

3b. Pipette 20 µL of the Lysis Solution for Blood onto the blood card punch. Samples can be spun in a microcentrifuge for a few seconds to force the solution into the punch.

4b. Incubate at 55 °C for 15 minutes.

5b. Add 180 µL of the Neutralization Solution for Blood. Mix thoroughly by vortexing or pipetting.

6b. Store the neutralized blood extract at 4 °C or use 2 µL immediately in PCR. Continue with step 7.
Note: DNA is stable in the extract for at least 6 months at 4 °C.

C. DNA Extraction from Cultured Mammalian Cells

1c. Grow monolayer cells in a multiwell plate until 90 to 95% confluent.

2c. Aspirate the medium from the wells using a pipette tip connected to the vacuum system. The medium must be removed completely.

3c. Add 20 µL of the Lysis Solution for Blood to the wells.
Note: It is preferred at this point to seal the plate with AlumaSeal™ II, Catalog Number A2350, to prevent loss by evaporation during incubation in step 4c. The Alumaseal can be pierced with a pipette tip to add the Neutralization Solution for Blood in step 5c. A new layer of AlumaSeal can be placed over the original layer to reseal the plate for storage.

4c. Incubate the plate at 75 °C for 5 to 10 minutes (for a 24 well plate, 5 minutes is recommended to avoid overdrying the samples).

5c. Add 180 µL of the Neutralization Solution for Blood to each of the wells. Mix the samples by pipetting up and down.

6c. Store the neutralized cell extract at 4 °C or use 2 µL immediately in PCR. Continue with step 7.
Note: DNA is stable in the extract for at least 6 months at 4 °C.

PCR amplification

The Extract-N-Amp Blood PCR ReadyMix contains the JumpStart antibody for specific hot start amplification. Therefore, PCR reactions can be assembled at room temperature without premature *Taq* DNA polymerase activity.

Typical final primer concentrations are approximately 0.4 µM each. The optimal primer concentration and cycling parameters will depend on the system used.

7. Add the following reagents to a thin-walled PCR microcentrifuge tube or plate:

Reagent	Volume
Water, PCR Reagent	x µL
Extract-N-Amp Blood PCR ReadyMix	10 µL
Forward primer	y µL
Reverse primer	y µL
Neutralized blood extract	2 µL
Total volume	20 µL

Note: The neutralized blood extract may inhibit PCR amplification of products larger than 2 kb. Neutralization Solution B, Catalog Number N3910, can be used overcome this inhibition and allows successful amplification of longer PCR products. Add 1 µL of Neutralization Solution B to each reaction. Neutralization Solution B is not part of this kit and must be purchased separately.

8. Mix gently.
9. For thermal cyclers without a heated lid, add 20 µl of mineral oil on top of the mixture in each tube to prevent evaporation.
10. Perform thermal cycling. The amplification parameters should be optimized for individual primers, template, and thermal cycler (see References for guidance).

Common cycling parameters:

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

11. The amplified DNA can be loaded onto an agarose gel after the PCR is completed with the addition of a separate loading buffer/tracking dye such as Gel Loading Solution, Catalog Number G2526.
    Note: PCR products can be purified, if desired, for applications such as sequencing with the GenElute™ PCR Clean-Up Kit, Catalog Number NA1020.

Related Products	Catalog Number
PCR 96 well plates	Z374903
PCR 384 well plates	P4606; Z37,491-1
Sealing mats and tape	P4481; Z374938
AlumaSeal II	A2350
EDTA, tripotassium salt dihydrate	E0270
PCR microtubes	Z374873; Z374962; Z374881
Collection Card	C2613
Neutralization Solution B	N3910
Mineral Oil	M8662
PCR Marker	P9577
Precast Agarose Gels	P6097
TBE Buffer	T4415; T6400; T9525
GenElute PCR Clean-Up Kit	NA1020
Gel Loading Solution	G2526

References

1. Dieffenbach, C.W., and Dveksler, G.S. (Eds.), PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1995). Catalog Number Z364118
2. Don, R.H. et al., 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res., **19,** 4008 (1991).
3. Erlich, H.A. (Ed.), PCR Technology: Principles and Applications for DNA Amplification, Stockton Press, New York (1989).
4. Griffin, H.G., and Griffin, A.M. (Eds.), PCR Technology: Current Innovations, CRC Press, Boca Raton, FL (1994). Catalog Number Z357499
5. Innis, M.A., et al., (Eds.), PCR Strategies, Academic Press, New York (1995). Catalog Number Z364452
6. Innis, M., et al., (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, California (1990). Catalog Number P8177
7. McPherson, M.J. et al., (Eds.), PCR 2: A Practical Approach, IRL Press, New York (1995). Catalog Number Z362387
8. Newton, C.R. (Ed.), PCR: Essential Data, John Wiley & Sons, New York (1995).
9. Roux, K.H. Optimization and troubleshooting in PCR. *PCR Methods Appl.*, **4,** 5185-5194 (1995).
10. Saiki, R., PCR Technology: Principles and Applications for DNA Amplification, Stockton, New York (1989).

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 and repeat PCR. To test for inhibition, include a DNA control and/or add a known amount of template (100-500 copies) into the PCR mixture along with the blood extract.
	A PCR component is missing or degraded.	Run a positive control to insure 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.
	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 denaturation temperature is too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time is too long or too short.	Optimize the denaturation time by increasing or decreasing the time in 10 second increments.
	The extension time is too short.	Increase the extension time in 1 minute increments, especially for long templates.
	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 templates at a concentration of 1.0-1.7 M.
Multiple products are seen.	JumpStart antibody is not working correctly.	Do not use DMSO or formamide with Extract-N-Amp PCR ReadyMix. It can interfere with the enzyme-antibody complex. Other solvents, salts, extremes in pH, or other reaction conditions may reduce the affinity of the JumpStart antibody for the *Taq* polymerase and thereby compromise its effectiveness.
	Touchdown PCR may be needed.	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications. Touchdown PCR uses an annealing/extension temperature that is higher than the T_m of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer T_m for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.
Negative control shows a PCR product or "false positive" results are obtained.	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 extraction or PCR are contaminated with a template from a previous reaction.

JC,RC,PHC 01/13-1