Product Information

Lysis Solution for Blood and Neutralization Solution for Blood

SRE0086, SRE0087

Product Description

The Lysis Solution for Blood and the Neutralization Solution for Blood are used together to rapidly extract and neutralize 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 for Blood 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 for Blood, the extract is now ready for PCR. The neutralized extract may be added directly to the PCR, at up to 10% final volume, without additional treatment. It may also be stored at 2–8 °C until ready for use.

Reagents

Sold Separately	1,000 preps	Catalogue Number
Lysis Solution for Blood	25 mL	SRE0086
Neutralization Solution for Blood	250 mL	SRE0087

Reagents and Equipment Required (Not Provided)

- Microcentrifuge tubes or multi-well plate for extractions (200 µL minimal volume)
- Punch and cards for dried blood
- Thermocycler, water bath, or oven for blood cards (55 °C), or dry block or oven for monolayer cells (75 °C)

Precautions and Disclaimer

For manufacturing, processing, or repacking. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the Lysis Solution for Blood and Neutralization Solution for Blood at room temperature. If either solution forms a precipitate, warm at 55–65 °C until the precipitate dissolves and cool to room temperature before use.

Stable for 18 months from the date of manufacture when stored at room temperature. Do not use past expiration date printed on product label.

Procedures

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

DNA extraction from Whole Blood

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 inversion or rocking.

   Note: For non-human sources, collect blood into tripotassium EDTA, Cat. No. E0270, at a final concentration of 5 mM to prevent coagulation.
2. Place 20 µL of the Lysis Solution for Blood into a microcentrifuge tube or well of a multi-well plate for each extraction.
3. Add 10 µL of blood. Mix thoroughly by vortexing or pipetting.
4. Incubate at room temperature for 5 minutes.
5. Add 180 µL of the Neutralization Solution for Blood. Mix thoroughly by vortexing or pipetting.
6. Store the neutralized blood extract at 2–8 °C or use up to 10% final volume immediately in PCR.

   Note: DNA is stable in the extract for at least 6 months at 2–8 °C.

DNA extraction from Blood Cards

1. Collect the blood sample onto a collection card, Cat. No.WHAWB100014. Allow to dry completely.
2. 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.
3. 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.
4. Incubate in a thermocycler or water bath at 55 °C for 15 minutes. If incubating in an oven, time should be extended 5–15 minutes to allow for proper heat transfer.
5. Add 180 µL of the Neutralization Solution for Blood. Mix thoroughly by vortexing or pipetting.
6. Store the neutralized blood extract at 2–8 °C or use up to 10% final volume immediately in PCR.

   Note: DNA is stable in the extract for at least 6 months at 2–8 °C.

DNA Extraction from Cultured Mammalian Cells

1. Grow monolayer cells in a 24- 96-well plate until 90–95% confluent.
2. Aspirate the medium from the wells using a pipette tip connected to the vacuum system. The medium must be removed completely.
3. Add 20 µL of the Lysis Solution for Blood to the wells. Swirl or tap plate to ensure complete coverage in the wells.

   Note: It is preferred at this point to seal the plate with AlumaSeal® II, Cat. No. A2350, to prevent loss by evaporation during incubation in step 4. The AlumaSeal® II can be pierced with a pipette tip to add the Neutralization Solution for Blood in step 5. A new layer of AlumaSeal® can be placed over the original layer to reseal the plate for storage.
4. Incubate the plate at 75 °C for 5–10 minutes (for a 24-well plate, 5 minutes is recommended to avoid over drying the samples).

   Note: When using an oven incubation time can be extended 5 minutes to allow for proper heat transfer.
5. Add 180 µL of the Neutralization Solution for Blood to each of the wells. Mix the samples by pipetting up and down.
6. Store the neutralized cell extract at 2–8 °C or use up to 10% final volume immediately in PCR.

   Note: DNA is stable in the extract for at least 6 months at 2–8 °C.

Troubleshooting Guide

Problem	Cause	Solution
Little or no PCR product is detected.	Extract was not neutralized	Make sure Cat. No. SRE0087, Neutralization Solution for Blood was added to the extract before PCR was performed.
	PCR reaction is inhibited due to contaminants in the blood extract.	Use less neutralized 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.
	Too few cycles are performed.	Increase the number of cycles (5–10 additional cycles at a time).
Color of extract	Color of the extract is a brownish tint.	The color of the extract should have a brownish/greenish tint after the addition of blood to the Lysis Solution for Blood and Neutralization Solution for Blood.
Negative control shows a PCR product, or "false positive" results are obtained.	Reagents are contaminated.	We recommend 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.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.