For life science research only.
Not for use in diagnostic procedures.

Print Protocol | Print Changes to Previous Version | Print Safety Precautions | Print

DNA Isolation Kit for Mammalian Blood

Version: 09
Content version: September 2017

For isolations from up to 10 ml mammalian blood.

Cat. No. 11 667 327 001 1 kit
25 purifications

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial	Label	Content
1	Red Blood Cell Lysis Buffer	750 ml
2	White Blood Cell Lysis Buffer	125 ml
3	Protein Precipitation Solution	65 ml

A white precipitate may be observed in the White Blood Cell Lysis buffer at lower temperatures. This precipitate will not affect the function of the kit. Dissolve the precipitate by warming the solution for approximately 15 min in a +37°C water bath, with occasional swirling.

1.2. Storage and Stability

Storage Conditions (Product)

Kit components are stable at +15 to +25°C until the expiration date printed on the label.

1.3. Additional Equipment and Reagents Required

- 70% Ethanol
- Ethanol
- 1x TE Buffer, pH 8.0 (optional)
- RNase* (optional)
- Glycogen* (optional)
- PBS* (optional)

1.4. Application

The DNA Isolation Kit for Mammalian Blood is designed for the rapid isolation of DNA from 1 – 10 ml mammalian whole blood, lymphocyte, or buffy coat samples. The purified DNA is suitable for multiple applications, including standard PCR, long-range PCR, sequencing, and Southern blots. The entire procedure can be completed in less than 1.5 h (plus 30 – 60 min resuspension time), requiring no organic extractions or column-purification steps. Multiple samples can be easily processed. The kit contains no hazardous organic solvents or chaotropic agents.

1.5. Preparation Time

Hands on time	Approximately 15 minutes
Total time	Approximately 1.5 hours plus resuspension time (for 4 samples)

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- Use 10 ml mammalian whole blood (*e.g.*, from human, mouse, rat). When using <10 ml blood, follow the alternate procedure titled, “Optimal procedure for use with smaller quantities of blood” for modifications to this general procedure.
- If blood was stored at +2 to +8°C or −15 to −25°C, warm the blood to +15 to +25°C prior to use.
- If using human blood components, such as lymphocytes or buffy coat, follow the alternative procedure titled, “Procedural modifications for use with buffy coat or lymphocyte samples.”
- Do not use blood that has been frozen and thawed more than 3 times, or yields will be significantly reduced.
- For best results, use fresh blood or blood stored for <3 days. Blood stored for 7 days at +2 to +8°C or <1 month at −15 to −25°C will result in a 10 –15% reduction in yield.
- Use sodium heparin-, sodium citrate-, or EDTA-treated blood. For heparin-treated blood, heat the white cell pellet in White Blood Cell Lysis Buffer for 10 minutes at +65°C to facilitate lysis.

General Considerations

Handling Instructions

⚠ ***Use sterile disposable polypropylene tubes and tips in order to avoid contamination.***
⚠ ***Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Properly dispose of all contaminated materials and decontaminate work surfaces and use a biosafety cabinet whenever aerosols might be generated.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.

2.2. Protocols

Procedure for DNA Isolation from 10 ml Mammalian Whole Blood

⚠ ***All procedures are performed at +15 to +25°C unless otherwise noted.***

Procedure

1. For each blood sample, add 30 ml Red Blood Cell Lysis Buffer to a sterile 50 ml centrifuge tube.

   Use a centrifuge tube that will withstand a minimum of 900 × g (preferable 12,000 × g), and accommodate a total volume of 40 ml.

---

2. – Add 10 ml mammalian blood to each tube.
   – Cap the tube, mix gently by inversion.

   ⚠ ***Do not vortex.***

---

3. Place the centrifuge tube on a rocking platform or gyratory shaker for 10 minutes.

   Alternatively, manually invert the sample periodically for 10 min.

---

4. Centrifuge the tube at 875 × *g* for 10 minutes (*e.g.* 2,000 rpm in a RT6000B Sorvall Centrifuge).

   Do not exceed centrifugation speed limit, this will complicate the resuspending of the white cell pellet (step 6).

---

5. Carefully pour off and properly dispose of the clear, red supernatant (indicative of full red cell lysis). Some residual liquid should remain with the white cell pellet.

   The Red Blood Cell Lysis Buffer selectively lyses the erythrocytes, leaving the leukocytes intact. Following centrifugation, if the sample appears as a cloudy upper layer (containing plasma/leukocytes) and a red lower layer (containing erythrocytes), no red cell lysis has occurred. If this happens, do one of the following:

   – Repeat steps 1 – 4 with fresh blood, using a 15 minute incubation in step 3.
   – Repeat steps 1 – 4 with fresh blood, inverting the sample more frequently if mixing by hand.
   – Ensure that fresh blood has been warmed to +15 to +25°C before repeating steps 1 – 4.

---

6. Thoroughly vortex the white pellet visible at the bottom of the tube in the residual supernatant.

   Vortex thoroughly, the white cell pellet must be completely resuspended in the residual supernatant. This step facilitates full lysis of the white cell pellet during step 7.

   The white cell pellet will still be slightly red in color due to the presence of residual hemoglobin. It will be removed in subsequent steps.

---

7. Add 5 ml White Cell Lysis Buffer, cap the tube, and vortex thoroughly to lyse the white cells completely.

   Vortex thoroughly to ensure the white cells are completely lysed. Following successful lysis of the leukocytes, the solution should appear clear dark red/brown with no particulate material present. To ensure this, perform an optional incubation at +37°C for 15–30 -min, which may facilitate lysis.

   For heparin-treated blood, heat the white cell pellet in White Blood Cell Lysis Buffer for 10 minutes at +65°C before vortexing.

---

8. **Optional:**
   – Add RNase* to a final concentration of 0.02 mg/ml.
   – Mix by inversion.
   – Incubate at +37°C for 15 – 60 minutes.

   RNase treatment completely removes RNA contaminants from the final DNA sample, yielding pure DNA. This is only recommended if subsequent applications require a RNA-free sample.

---

9. Transfer the sample to a sterile 17 × 100 mm tube or continue using the 50 ml centrifugation tube if it is capable of withstanding 12,000 × *g* centrifugal force.

---

10 Add 2.6 ml Protein Precipitation Solution to each sample.

Vortex thoroughly for approximately 25 seconds to remove protein from the sample.
Upon vortexing, a brownish protein precipitate will be clearly visible.

---

11 Centrifuge the sample at 12,000 × *g* (*e.g.* 10,000 rpm in a Sorvall RC5B centrifuge) for 10 minutes.

Lower speed spins will result in loose protein pellets, making it very difficult to effectively separate the proteinaceous material from the supernatant.

---

12 – Carefully pour the supernatant, which contains the DNA, into a new sterile 50 ml centrifuge tube.
– Properly dispose of the protein pellet.

The new centrifuge tube must withstand a minimum of 900 × g (or 12,000 × g for isolation of <1.5 × 10^7 cells).

---

13 Ethanol precipitate the DNA:
– Add 2 volumes of +15 to +25°C ethanol to the supernatant from step 12.
– Gently mix by inversion until DNA strands precipitate out of solution and the remaining liquid is no longer cloudy.
– Centrifuge the sample at 875 × *g* for 10 min (*e.g.*, 2,000 rpm in a Sorvall RT6000B centrifuge).
– Discard the supernatant.

For <1.5 x 10^7 cells, centrifuge at 12,000 × g for 10 min following addition of glycogen carrier.

Optional Method:
Instead of centrifugation, a sterile blunt-ended glass rod may be used to carefully remove the DNA strands from the 100% ethanol before transferring them to a new sterile tube containing cold 70% ethanol (see step 14). Swirl until DNA strands are released into 70% ethanol.

If the number of leukocytes in the sample is less than 1.5 × 10^7 cells, the yield may be insufficient to see visible DNA strands falling out of solution. If this occurs, add glycogen carrier prior to the ethanol precipitation, and increase the centrifugal to 12,000 × g to facilitate effective precipitation.*

---

14 – Add 3 ml cold 70% ethanol to the DNA pellet.
– Mix the sample several times by gentle inversion.
– Centrifuge the sample at 875 × *g* for 5 minutes (*e.g.* at 2,000 rpm in a Sorvall RT6000B centrifuge).
– Discard the supernatant.

⚠ ***Do not vortex during this wash step.***
For samples containing <1.5 × 10^7 cells, centrifuge the DNA at 12,000 × g for 5 minutes after the 70% ethanol wash.

---

15 Dry the DNA pellet by placing the sample under vacuum without heat for a few minutes or until the ethanol is no longer visible.
Or:
Allow the sample to air dry.

Do not overdry the DNA pellet, this may lead to difficulties in fully resuspending the DNA.

---

16 – To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0, or desired buffer.
– Vortex thoroughly.
– Place samples at +65°C for 30 minutes (human blood samples) / 60 minutes (other mammalian samples), periodically vortex thoroughly.

---

17 Store samples at +2 to +8°C until use.

If desired, samples can be accurately quantified using spectrophotometry or fluorometry.

Using the isolated DNA

DNA prepared using the DNA Isolation Kit for Mammalian Blood can be effectively used in multiple applications, including Southerns and PCR with either Taq DNA polymerase or Expand PCR System* products. Once quantified, use the same amount of DNA per application as you would typically use of DNA prepared with an alternative purification method.

Optional procedure for use with smaller quantities of blood

By slightly adjusting the procedure detailed in the section titled "DNA isolation from 10 ml mammalian whole blood" blood samples from 1 – 10 ml can be processed. Follow the procedure described above with the following volume modifications:

Blood Volume [ml]	Red Blood Cell Lysis Buffer Volume: Blood Ratio* (Step 1)	White Blood Cell LysisBuffer Volume [ml] (Step 7)	Protein Precipitation Solution volume [ml] (Step 10)	Recom. Resuspension Volumes [µl] (Step 16)
9 – <10	3:1	5.0	2.6	1000
8 – <9	3:1	5.0	2.6	800
7 – <8	3:1	5.0	2.6	800
6 – <7	3:1	5.0	2.6	600
>5 – 6	3:1	5.0	2.6	600
4 – 5	3:1	2.5	1.3	400
3 – <4	3:1	1.5	0.780	400
2 – <3	3:1	1.0	0.520	200
1 – <2	3:1	1.0	0.520	200

*Add 3 ml Red Blood Cell Lysis Buffer for every 1 ml whole blood.

Procedural Modification for Use with Lymphocyte Samples or Buffy Coat

Freshly isolated lymphocyte samples or buffy coat can be used as starting material for the purification of DNA by slightly adjusting the procedure detailed in the section titled "DNA isolation from 10 ml mammalian whole blood."

For Lymphocytes

1. Isolate the lymphocyte population from 10 ml human blood, following the procedure outlined in the Instructions for Use provided with the lymphocyte separation medium you choose (*e.g.* using standard Ficoll-Hypaque density gradients).

2. Wash the lymphocyte population twice with sterile 1× PBS prior to use.

3. At this point, an aliquot may be removed to determine cell counts.

4. Once the lymphocyte cell pellet is prepared, proceed directly to step 7 of the section titled "DNA isolation from 10 ml mammalian whole blood" modifying the procedure by adding 2.5 ml White Blood Cell Lysis Buffer per sample.

5. Adjust the subsequent steps accordingly (*e.g.* use 1.3 ml Protein Precipitation Solution in step 10). Resuspension of the DNA in 500 µl TE, pH 8.0, is recommended as a starting point.

   Average yields range from 75–300 µg / 1.1 - 4.2 × 10^7 cells

For Buffy Coat

1. Prepare the buffy coat from 10 – 20 ml human blood by placing the sample at +15 to +25°C for 30 minutes or at +2 to +8°C overnight to allow the phases to separate. Alternatively, the blood may be centrifuged at 1,300 × *g* for 15 minutes at +15 to +25°C. The buffy coat is the interface between the plasma-containing upper phase and erythrocyte-containing lower phase.

---

2. Once phase separation has occurred, carefully remove and discard the upper plasma phase with a sterile Pasteur pipette, exposing the buffy coat layer.

---

3. Transfer the buffy coat to a sterile 17 × 100 mm tube capable of withstanding a centrifugal force of 12,000 × *g*. Be careful not to remove any of the erythrocyte layer with the buffy coat. At this point, an aliquot may be removed to determine cell count. If the total number of leukocytes isolated is less than 1.0×10^7, the procedure will not work effectively. Obtain more blood sample, and start the procedure again.

---

4. – To the isolated buffy coat, add 5 ml sterile 1× PBS, mix gently by inversion, and centrifuge at 875 × *g* for 10 minutes at +15 to +25°C.
   – Discard the supernatant.

---

5. Once the white cell pellet is obtained, proceed directly to step 7 of the section titled “DNA isolation from 10 ml mammalian whole blood” modifying the procedure by adding 1.5 ml White Blood Cell Lysis Buffer per sample. Adjust the subsequent steps accordingly (*e.g.* use 780 µl Protein Precipitation Solution in step 10). Resuspension of the isolated DNA in 300 µl TE, pH 8.0, is recommended as a starting point.

   Average yields range from 35–105 µg/1.1 - 2.3 × 10^7 cells

---

3. Results

Yield

Yields can be determined via spectrophotometry or fluorometry. Average yields are approximately 350 µg / 10 ml, ranging from 200 – 700 µg for healthy human blood (average, 5 × 10^6 leukocytes/ml). Note that the amount of DNA recovered will vary significantly depending on the number of white cells present in the donor blood.

Average Yields

Refer to the following table for average DNA yields from the tissue of various species.
The A_{260}/A_{280} ratio for isolated DNA samples is typically 1.7 – 1.9.

Species	Average yield [µg/10 ml blood]	Yield range [µg]
Mouse	570	460 - 670
Rat	580	350 - 680
Dog	450	350 - 600
Porcine	670	520 - 780
Guinea pig	160	55 - 295

4. Troubleshooting

Observation	Possible cause	Recommendation
No red blood cell lysis	If the sample appears as a cloudy upper layer (containing plasma/ leukocytes) and a red lower layer (containing erythrocytes) following centrifugation, no red cell lysis has occurred.	Repeat steps 1 – 4 with fresh blood, using a 15-minute incubation in step 3.
		Repeat steps 1 - 4 with fresh blood, inverting the sample more frequently if mixing by hand.
		Be certain to warm the blood to +15 to +25°C and repeat steps 1 – 4.
Incomplete white blood cell lysis	Evident by particulate material present in sample following vortexing (see step 7).	Prior to addition of White Blood Cell Lysis Buffer, vortex the white cell pellet thoroughly, making sure that the white cell pellet is fully resuspended in the residual volume. This step facilitates full lysis of the white cell pellet in step 7. If this is not done, it will be very difficult to completely resuspend the white cell pellet in White Blood Cell Lysis Buffer.
		Vortex the sample thoroughly following addition of the White Blood Cell Lysis Buffer to ensure that the white cells are completely lysed.
		Increase the volume of White Blood Cell Lysis Buffer to accommodate a larger number of white cells. If too many cells are present, the solution will become very viscous, and the cells will clump.
		To facilitate full lysis, samples may be incubated at +37°C for 15 – 30 minutes.
No protein pellet is observed following protein precipitation	If the leukocyte number is small ($<1 \times 10^7$ cells), the protein pellet may be visible as a small, tan / brown or clear pellet. When using lymphocytes or buffy coat starting material, the protein pellet will be clear.	Mix the sample thoroughly by vortexing after addition of the Protein Precipitation Solution. Recommendation: Vortex continuously for approximately 25 seconds.
		To ensure effective pelleting of protein, the samples must be spun at 12,000 × *g* for a minimum of 10 minutes. Lower speed spins will result in loose protein pellets, which make it very difficult to effectively separate the protein from the supernatant.
DNA resuspension: samples are slow to rehydrate	Samples were overdried prior to resuspension.	Air dry the samples, which helps to reduce overdrying. This method requires a longer time to finish.
		Heat to +65°C to aid resuspension. Do not exceed 1 h incubation time at +65°C. Alternatively, resuspend samples overnight at +2 to +8°C.
		Do not exceed 5 min of drying time under vacuum, and do not use heat when drying

Observation	Possible cause	Recommendation
Low DNA yields	There was an insufficient number of leukocytes in the starting sample.	Increase the volume of starting sample.
	Incomplete white blood cell lysis.	See recommendations above (under “Incomplete white blood cell lysis”).
		If using blood stored for 7 days at +2 to +8°C or ≤1 month at −15 to −25°C, the expected yields will be 10 –15% lower than those of freshly isolated blood.
		Adjust the volume of lysis buffer to accommodate smaller numbers of leukocytes or smaller volumes of blood.
DNA is not functional in subsequent applications (e.g., the $A_{260/280}$ ratio too low or too high)	A_{260}/A_{280} <1.6: Protein contamination is present.	Follow recommendations above (under “No protein precipitation”). Check to make sure the DNA is completely in solution.
	A_{260}/A_{280} <2.0: RNA contamination is present	Repeat RNase treatment, and reprecipitate the DNA. Increase the incubation time for RNase treatment from 15 – 30 min at +37°C.
		Quantify the DNA prior to initiating subsequent applications. Use the same amount of DNA per application as you would typically use of DNA prepared with an alternative purification method.

5. Additional Information on this Product

5.1. Test Principle

How this Product Works

- Isolation of DNA from whole blood can be difficult because blood is a complex mixture containing cells, proteins, metabolites, etc. Most of the cells (>99%) are erythrocytes (red blood cells), which lack nuclei, and therefore, possess no DNA. Only leukocytes (0.3% of total blood cells), also called white blood cells, contain nuclei and DNA. Therefore, DNA from blood must be isolated from one of three types of leukocytes: monocytes, lymphocytes (25% of the leukocyte population), or granulocytes (Alberts et al., 1989).
- The DNA Isolation Kit for Mammalian Blood procedure relies on separation of the white blood cells from whole blood via a preferential red blood cell lysis. In the presence of a strong anionic detergent, the white blood cells are then lysed, and the proteins removed by dehydration and precipitation. The purified DNA is subsequently recovered via ethanol precipitation (Miller et al., 1993, Lahiri and Schnabel, 1993).

5.2. References

- Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson J - (1989) , 973-976
- Lahiri DK, Schnabel B - DNA isolation by a rapid method from human blood samples: Effects of MgCl2, EDTA, storage time, and temperature on DNA yield and quality (1993) *Biochemical Genetics* 7, 321-328
- Miller SA, Dykes DD, Polesky HF - A simple salting out procedure for extracting DNA from human nucleated cells (1988) *Nucleic Acids Research* 3, 1215-

5.3. Quality Control

The following table describes the quality control performed on each kit:

Tested for...	
Absence of DNase contamination	Each lot of the DNA Isolation Kit for Mammalian Blood is tested for absence of DNase contamination. The Red Blood Cell Lysis Buffer, White Blood Cell Lysis Buffer, and Protein Precipitation Solution are each incubated with 1 µg pBR322 DNA for 6 hours at +37°C. The DNA is then visualized by electrophoresis on an agarose gel and compared to a positive control to determine if any linear or nicked plasmid DNA is visible.
DNA isolation and amplification	Each lot of kits is function tested for the ability to purify DNA from human whole blood, followed by specific amplification of a 4.8 kb tPA fragment via PCR with the Expand Long Template PCR System*. The 4.8 kb tPA product is visualized by electrophoresis on an agarose gel, and two samples are compared with a positive control of human genomic DNA to determine if the same size amplification product is obtained. An intense, single 4.8 kb tPA band is visible.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents , kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
RNase, DNase-free	500 µg, 1 ml	11 119 915 001
Expand Long Template PCR System	150 U, 5 U/µl, 38 reactions in a final volume of 50 µl	11 681 834 001
	720 U, 2 x 360 U, 190 reactions in a final volume of 50 µl	11 681 842 001
	3,600 U, 10 x 360 U, 950 reactions in a final volume of 50 µl	11 759 060 001
Expand High Fidelity PCR System	100 U, 3.5 U/µl, 40 reactions in a final volume of 50 µl	11 732 641 001
	500 U, 2 x 250 U, 200 reactions in a final volume of 50 µl	11 732 650 001
	2,500 U, 10 x 250 U, 1,000 reactions in a final volume of 50 µl	11 759 078 001
Expand 20 kb^{PLUS} PCR System	200 U, 5 U/µl, 40 reactions in a final volume of 50 µl	11 811 002 001
Glycogen	20 mg, 1 ml	10 901 393 001

6.4. Trademarks

EXPAND is a trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country to display country-specific contact information.

0917.04992601001©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany