



DNA Isolation Kit for Mammalian Blood

for isolation of genomic DNA from 25 samples (10 ml each)

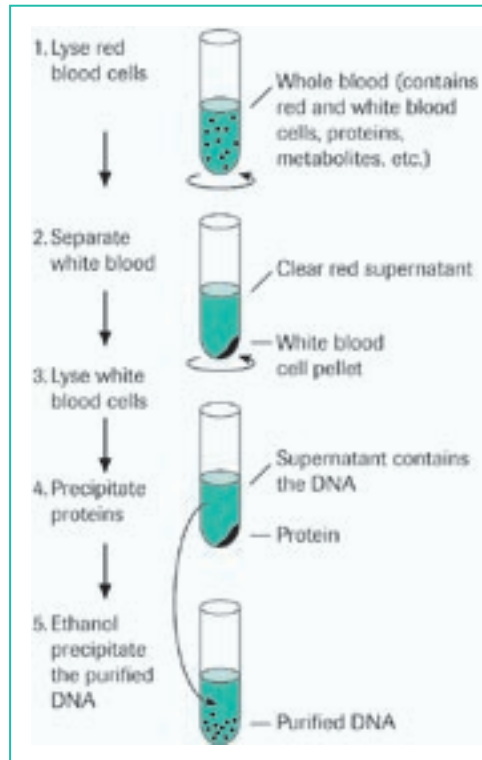
Cat. No. 11 667 327 001

Principle	The kit procedure starts with preferential lysis of erythrocytes. The remaining leukocytes are lysed with a strong anionic detergent, and proteins are removed by dehydration and precipitation. The purified genomic DNA is then recovered via ethanol precipitation.
Starting material	<ul style="list-style-type: none"> ● 1 – 10 ml whole blood (research samples) that has been treated with an anticoagulant (sodium heparin, sodium citrate, or EDTA) ●  <i>The kit works best with fresh blood or blood stored (at 2 to 8°C or –15 to –25°C) for ≤3 days. It should not be used with blood that is more than 1 month old or blood that has been frozen and thawed more than 3 times.</i> ● Lymphocyte preparations from 10 ml mammalian blood (research samples) ● Buffy coat preparations from 10 – 20 ml mammalian blood (research samples)
Application	<ul style="list-style-type: none"> ● For preparation of high molecular weight, purified genomic DNA, which may be used in standard and long-template PCR, Southern blots, etc. ●  <i>The kit has been used to prepare DNA from human, mouse, rat, dog, porcine, guinea pig, monkey, rabbit, and bovine blood.</i>
Time required	<ul style="list-style-type: none"> ● Total time: approx. 90 min (plus 30 – 60 min to resuspend DNA) ● Hands-on time: <50 min
Results	<p>Yield: Variable, depending on species (See the table under Part IV of “How to use the kit” in this article)</p> <p>Purity: Average A_{260}/A_{280} of isolated DNA = 1.7 – 1.9</p>
Benefits	<ul style="list-style-type: none"> ● Saves time, because the kit can prepare DNA directly from whole blood without prior isolation of leukocytes or buffy coat ● Increases lab efficiency, because the kit can prepare multiple DNA samples, free of RNA and protein, in approx. 90 min ● Increases lab safety, because the kit does not require extensive handling of potentially hazardous samples, nor use hazardous organic reagents ● Accommodates “real world” samples with varying amounts of leukocytes, because the sample volume can be adjusted from 1 – 10 ml

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How to use the kit

I. Flow diagram



II. Kit contents

- Red Blood Cell Lysis Buffer (750 ml)
- White Blood Cell Lysis Buffer (125 ml)
- Protein Precipitation Solution (65 ml)

III. Additional materials needed

- Absolute ethanol, at 15 to 25°C
- 70% ethanol, cold
- Tris-EDTA (TE) buffer, pH 8.0 (or other suitable DNA storage buffer)
- Sterile centrifuge tubes that can hold at least 40 ml (preferably 50 ml) and withstand a centrifugation force of 900 x g
- Sterile 17 x 100 mm tubes that can withstand a centrifugation force of 12,000 x g
- RNase (for optional digestion step)

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IV. Expected DNA yield from different mammalian whole blood sources

During testing of this kit, we obtained the following yield data when starting with samples of whole blood that were ≤ 3 days old:

Species	Average yield ($\mu\text{g}/10$ ml blood)	Yield range ($\mu\text{g}/10$ ml blood)
Dog	450	350 – 600
Guinea pig	160	55 – 295
Human	350	200 – 700
Mouse	570	430 – 670
Pig	670	520 – 780
Rat	580	350 – 680



The average A_{260}/A_{280} ratio of the isolated DNA was 1.7 – 1.9.



Starting with blood that had been stored for 7 days at 2 to 8°C or ≤ 1 month at -15 to -25 °C will lead to a 10 – 15% reduction in yields.

V. Protocol for preparing DNA from 10 ml samples of whole blood

General notes:

- ▶ To prepare DNA from buffy coat, lymphocytes, or blood volumes < 10 ml, modify this protocol as detailed in the package insert supplied with the kit.
- ▶ Before you start preparing the DNA, be sure to:
 - ▶ Warm all solutions from the kit to 15 to 25°C.
 - ▶ Warm blood samples to 15 to 25°C.

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

2 To each tube:

- ▶ Add 10 ml mammalian whole blood.
- ▶ Cap the tube and gently mix by inversion.







Do not vortex tubes.

3 Mix the tubes by:

- ▶ EITHER placing the centrifuge tube on a rocking platform or gyratory shaker for 10 min.
 - ▶ OR inverting the sample at regular intervals by hand, for a total of 10 min.
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- 4 After the incubation:
- ▶ Centrifuge the tube for 10 min at 875 x *g*.
 - ▶  *Do not centrifuge the tube at more than 875 x g.*
 - ▶ Look at the supernatant. Is it clear and red?
 - ▶ If yes, red cells have lysed completely. Go to Step 5.
 - ▶ If no, red cells did not lyse. Stop the protocol; see Part VI below.
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- 5 Carefully pour off and discard the clear, red supernatant.
- ▶  *Leave some supernatant with the white cell pellet.*
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- 6 Vortex the tube to thoroughly resuspend the white cell pellet in the residual supernatant.
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- 7 For each sample:
- ▶ Add 5 ml White Cell Lysis Buffer, then cap the tube.
 - ▶ Mix the contents of the tube thoroughly by vortexing.
 - ▶  *After vortexing, the solution should be dark brown or red, and contain no undissolved particles.*
 - ▶ Did the starting sample contain heparin?
 - ▶ If no, skip this step and go directly to Step 8.
 - ▶ If yes, heat the contents of the tube to 65°C for 10 min to ensure complete white cell lysis.
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- 8 Do you need the final DNA preparation to be free of RNA?
- ▶ If no, skip this step and go directly to Step 9.
 - ▶ If yes, remove all RNA from the DNA preparation as follows:
 - ▶ To the tube containing DNA, add 0.02 µg RNase per µl DNA solution.
 - ▶ Mix the tube gently by inversion.
 - ▶ Incubate the tube at 37°C for 15 min.
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- 9 Transfer the sample to a sterile 17 x 100 mm centrifuge tube.
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- 10 For each sample:
- ▶ Add 2.6 ml Protein Precipitation Solution to the tube.
 - ▶ Vortex the tube thoroughly (for approximately 25 s).
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- 11 Centrifuge the tube for 10 min at 12,000 x *g*.
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- 12 Carefully pour the supernatant, which contains the DNA, into a new sterile 50 ml centrifuge tube, then:
- ▶ Add 2 volumes of absolute ethanol 15 to 25°C.
 - ▶ Cap the tube.
 - ▶ Gently mix the contents of the tube by inversion until the DNA strands precipitate and the remaining liquid is no longer cloudy.
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- 13 Collect the visible strands of DNA by either centrifugation or by spooling:
- ▶ If you want to collect the DNA by centrifugation, then:
 - ▶ Centrifuge the tube for 10 min at 875 x *g*.
 - ▶ Discard the supernatant.
 - ▶ Add 3 ml of cold 70% ethanol to DNA pellet and resuspend by inversion.
 - ▶  *Do not vortex.*
 - ▶ Go to Step 14.



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- ▶ If you want to collect the DNA by spooling, then:
 - ▶ Wind the DNA strands around a sterile, blunt-ended glass rod.
 - ▶ Dip the glass rod containing the DNA into 3 ml cold 70% ethanol in a new sterile 50 ml centrifuge tube.
 - ▶ Swirl the glass rod until the DNA strands are released into the 70% ethanol.
 - ▶ Go to Step 14.


14 Centrifuge the tube containing 70% ethanol and DNA for 5 min at 875 x *g*. Discard the supernatant.

15 Dry the DNA pellet by:


- ▶ EITHER placing the tube under vacuum **without heat** until the ethanol is no longer visible (usually <5 min).
- ▶ OR inverting the tube and allowing the sample to air dry.

16 To rehydrate and dissolve the DNA pellet:

- ▶ Add 1 ml of Tris-EDTA (pH 8.0) (other buffer) to the tube.
- ▶ Vortex thoroughly.
- ▶ Incubate at 65°C for 30 – 60 min.

 For DNA from human blood, use a 30 min incubation. For DNA from other mammalian species, use a 60 min incubation.

- ▶ During the incubation, periodically vortex the tubes to facilitate solubilization of the DNA.

 Do not incubate the tube for >60 min at 65°C.

- ▶ Store DNA at 2 to 8°C.

VI. Troubleshooting the DNA Isolation protocol

If you get...	Then, the cause may be...	And you should...
A cloudy upper layer and a red lower layer at Step 4	Little or no red cell lysis	<ul style="list-style-type: none"> ▶ Start with fresh samples and repeat Steps 1 – 4 of the protocol with one or more changes: ▶ Be sure blood is at 15 to 25°C before starting protocol. ▶ Use a 15 min incubation in Step 3. ▶ If mixing by hand in Step 3, invert the tube more frequently.
Particles present in sample after vortexing (Step 7)	Incomplete white cell lysis	<p>Using fresh samples, repeat Protocol Va, with one or more changes:</p> <ul style="list-style-type: none"> ▶ In Step 6, be sure the white cell pellet is fully resuspended in the residual supernatant after vortexing. ▶ After adding buffer in Step 7, vortex the sample until the solution is dark brown/red and contains no particles. ▶ In Step 7, add enough White Blood Cell Lysis Buffer to ensure the solution is not viscous and does not contain clumps of cells. ▶ In Step 7, incubate the tube at 37°C for 15 – 30 min to facilitate lysis.



VI. Troubleshooting the DNA Isolation protocol, continued

If you get...	Then, the cause may be...	And you should...
Little or no protein pellet after Step 10	Ineffective protein precipitation	Using fresh samples, repeat Protocol Va, with one or more changes: <ul style="list-style-type: none"> ▶ In step 10 vortex the tube for approximately 25 s after adding Protein Precipitation Solution. ▶ Centrifuge the tube at 12,000 x g for at least 10 min.
	Very few leukocytes in sample	▶ Do nothing. A clear or small brown precipitate is normal in Step 10 if the starting blood sample contained $<10^7$ leukocytes.
DNA that is difficult to dissolve	Precipitated DNA was overdried in Step 15	▶ In Step 16, let the DNA rehydrate and dissolve overnight at 2 to 8°C.
Low DNA yields	Too few leukocytes in sample	Repeat Protocol Va, with one or more changes: <ul style="list-style-type: none"> ▶ Use a larger volume of blood (up to 10 ml) as the starting sample. ▶ In Step 7, use less lysis buffer.
	Incomplete white cell lysis occurred in Step 7	▶ Follow the troubleshooting recommendations under “Particles present in sample after vortexing” above.
	Starting blood sample too old	▶ Repeat protocol, but use fresh blood or blood that has been stored <3 days at 2 to 8°C or -15 to -25 °C.
DNA which does not work in downstream applications	Protein contamination present in DNA ($A_{260}/A_{280} < 1.6$)	▶ Follow troubleshooting recommendations under “Little or no protein pellet after Step 10” above.
	RNA contamination present in DNA ($A_{260}/A_{280} > 2.0$)	▶ Repeat RNase treatment (Step 8), then reprecipitate DNA. ▶ Increase the RNase incubation time to 30 min.
	Concentration of DNA not optimal for application	▶ Determine the amount of DNA in the preparation, then use the same amount of DNA in the application that you would normally use if you prepared DNA by another purification method.

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Typical results with the kit

Experiment 1

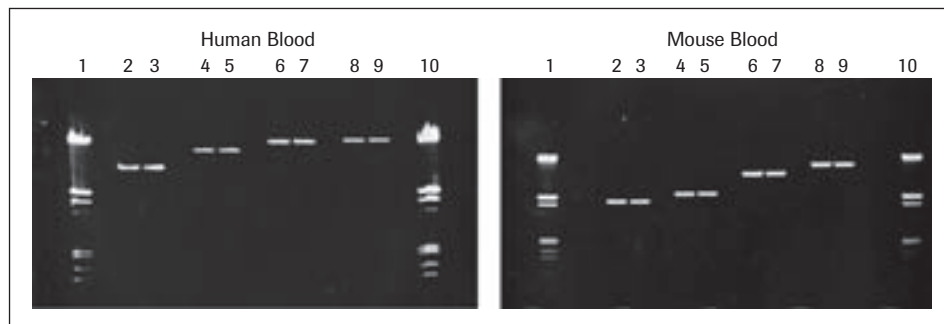


Figure 41: Use of DNA from human and mouse blood (prepared with the DNA Isolation Kit for Mammalian Blood) as templates for the amplification of large DNA fragments. Human and mouse blood research samples were collected as previously described (Noeth and Dasovich-Moody, 1997). DNA was prepared from 10 ml of each sample according to the protocol. Aliquots of each DNA preparation were used as templates for the long-template PCR amplification of several gene fragments. Each amplification procedure was performed with the Expand Long Template PCR System, using instructions given in the package insert.

Left panel shows gene fragments amplified from human DNA:

Lanes 1, 10: DNA Molecular Weight Marker III

Lanes 2, 3: tPA fragment (9.3 kb) amplified from 25 ng DNA

Lanes 4, 5: tPA fragment (15 kb) amplified from 50 ng DNA

Lanes 6, 7: β -globin fragment (23 kb) amplified from 100 ng DNA

Lanes 8, 9: β -globin fragment (28 kb) amplified from 200 ng DNA

Right panel shows the following fragments amplified from mouse DNA:

Lanes 1, 10: DNA Molecular Weight Marker III

Lanes 2, 3: IL-2 gene (4.2 kb) amplified from 330 ng DNA

Lanes 4, 5: α -2 collagen fragment (5.6 kb) amplified from 100 ng DNA

Lanes 6, 7: α -2 collagen fragment (10.4 kb) amplified from 50 ng DNA

Lanes 8, 9: α -2 collagen fragment (15.4 kb) amplified from 100 ng DNA

Result: All fragments were clearly visible on gels.

Experiment 2

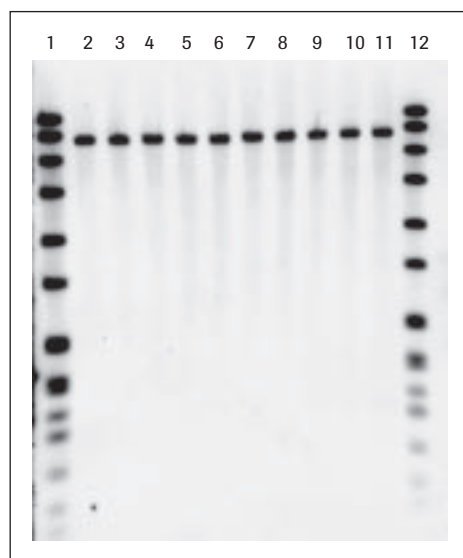


Figure 42: Use of DNA from various human blood research samples (prepared with the DNA Isolation Kit for Mammalian Blood) for detection of the n-ras gene by Southern hybridization.

The above protocol was used to prepare DNA from several human blood samples, each of which had been prepared with a different anticoagulant. DNA was also prepared from a lymphocyte preparation and a buffy coat preparation, using a modification of the protocol outlined in the kit package insert. Ten μ g of each preparation was digested with Eco RI, electrophoretically separated, and blotted to a nylon membrane. DNA on the membrane was hybridized to a DIG-labeled n-ras probe, and the results visualized chemiluminescently. The samples used were:

Lanes 1, 12: DNA Molecular Weight Marker VII

Lanes 2, 3: whole blood, sodium citrate anticoagulant

Lanes 4, 5: whole blood, heparin anticoagulant

Lanes 6, 7: whole blood, sodium EDTA anticoagulant

Lanes 8, 9: buffy coat preparation

Lanes 10, 11: lymphocyte preparation

Result: Each lane contained only a single hybridization band of the expected (7.2 kb) size (Taparowsky et al., 1983).

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References

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