Distance Learning

Issue

The Applied Science area went through a period-of-time when there were many, many new products. It was difficult for the sale people to learn about the products as well as have the salient information at their fingertips.

Solution

I created a unique blend of training and reference tool for each major new product. The reference information was product basics such as:

- catalog number
- price
- pack size(s)
- storage conditions
- shipping conditions
- priority
- marketing manager
- availability date

Additionally, it helped people with a limited science background as well the people with a lot of science experience. By the same token, it provided objectives, features, benefits and questions to ask customers. Other information that was included, when available were:

- pack insert
- marketing data
- newsletter article
- target customers
- associated techniques for extra sales
- competitors and their pricing
- illustrations of technique

Results

The salespeople were able to sell the new products and compile a body of knowledge for future use.

See the 2 examples on the next 21 pages:

- High Pure PCR Template Preparation Kit
- DNA Isolation Kit for Mammalian Blood.

Classifications

The table below lists three classifications for this work sample.

Learning Styles	**Intelligences[1]**	**Example of [2]**
Visual	Intrapersonal	Distance learning
Solitary	Visual-Spatial	

[1] Intelligences refers to Gardner's Multiple Intelligences.

[2] Reason(s) it was provided as a work sample

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High Pure PCR Template Preparation Kit

Catalog #	Pack Size	$ Price	Priority	Product Class
1796 828	100 rxns	$140.00	A *Key focus product/Me-Too product*	18 *Columns*

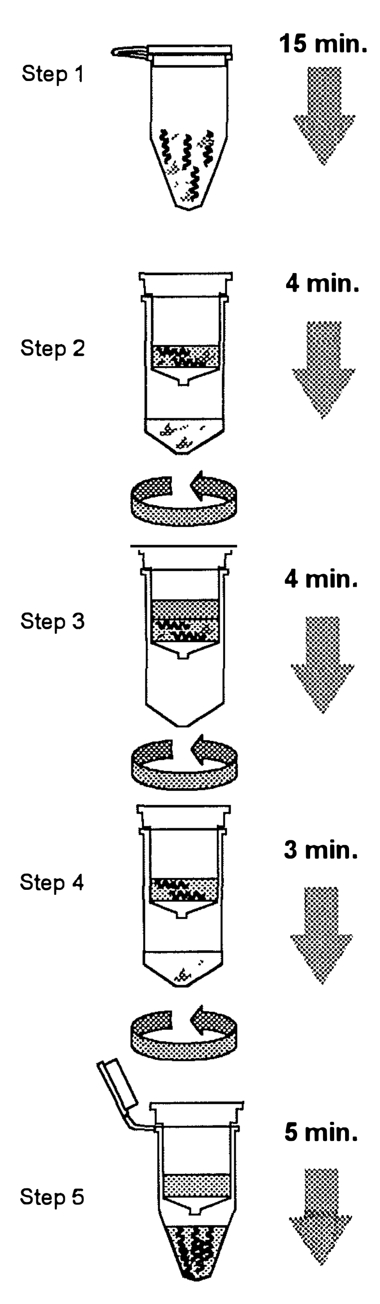
What Does It Do and How Does It Work?

The latest addition to our High Pure line, the High Pure PCR Template Preparation Kit does exactly what it says-purifies DNA to be used as a template for PCR. Similar to the other products in the High Pure family, this kit is based on the binding and eluting of nucleic acids to glass fiber fleece in spin filter tubes. In the presence of a chaotropic salt (in the tissue lysis buffer), DNA will be bound to the glass fibers [Steps 1 & 2]. The immobilized DNA can then be washed [Step 3] to remove impurities and then eluted with a Tris buffer [Step 4]. This product differs from the other High Pure Kits in that it is designed for the *isolation* [Step 1] as well as purification of genomic DNA. This kit includes components for isolation of DNA from a variety of starting materials such as whole blood, cultured cells, tissue specimens, bacteria and yeast. Isolation of genomic DNA from yeast or bacteria requires pretreatment with lysozyme or lyticase prior to using the kit. The procedure yields highly pure DNA suitable for PCR or digestion with restriction enzymes followed by Southern blot analysis. The total time required for the procedure is ≤35 minutes when whole blood or cultured cells are used as starting material, ≤95 minutes for tissue, ≤80 minutes for yeast and ≤ 65 minutes for bacteria samples. The yields vary from 3-10 µg, depending on the type of sample used in the procedure. Flow diagrams of the procedures for each sample type can be found under **Extra Information**.

Companion products would be: Expand™ Hi-Fi, Expand™ LT, Taq, Random Primed Labeling Kit, Genius System, and cloning products such as restriction enzymes and Rapid DNA Ligation Kit.

Summary

- Purification principle based on binding of DNA to glass fiber fleece in the presence of a chaotropic salt (guanidine thiocyanate)
- Used to isolate analytical quantities of genomic DNA from whole blood, cultured cells, tissue specimens, bacteria and yeast (for bacteria and yeast, pretreatment with lyticase or lysozyme is necessary).
- Yields genomic DNA suitable for PCR or Southern blotting



Step 1-Mix sample with Binding Buffer, add proteinase K, and incubate. **Step 2**-Mix with isopropanol, and transfer to filter tube. **Step 3**-Discard flowthrough, and add Washing Buffer. **Step 4**-Repeat washing step. Spin 10s high speed. **Step 5**-Elute nucleic acid.
TOTAL TIME: <35 min.
(Hands-on time: <20 min.)

Who Should I Talk To?

Researchers isolating DNA from whole blood, cultured cells, tissue specimens, bacteria and yeast. Some **buzzwords** would be isolating DNA, human blood, bacteria, isolating DNA from tissue, midi prep, maxi prep, QIAamp or Wizard (competitor's kits), PCR, Southern blot, cloning.

Techniques Associated with Product	Contents (Physical Description)*
DNA Extraction Methods: Phenol /Chloroform CsCl Protease/Column techniques **Downstream applications:** PCR Cloning Southern Blot Sequencing Mutation analysis-CFLP, RFLP, SSCP, DGGE	1. **Tissue lysis buffer**, white cap (20 ml) 2. **Binding buffer**, green cap (20 ml) 3. **Proteinase K**, lyophilizate (90 mg)- Dissolve in 4.5 ml Di H_2O, aliquot and store at -20°C. 4. **Washing buffer**, blue cap (20 ml) *NOTE: Customer must add 80 ml of ethanol before using this reagent!* 5. **Elution buffer**, colorless cap (40 ml) *NOTE: Elution buffer must be pre-warmed to 70°C in order to work effectively* 6. **High Pure filter tubes**-2 bags, each with 50 tubes-Polypropylene tubes with two layers of glass fiber fleece. Capacity is 700µl max. 7. **Collection tubes**-2 bags, each with 50 2 ml polypropylene tubes * Details about buffer composition can be found under **Extra Information** below or in the pack insert.

Additional Materials and Reagents Required

All Sample Types:	For blood and cultured cells: / For yeast:	For tissue: / For bacteria:
Biological sample (i.e. blood, bacteria, yeast, cultured cells or tissue) Ethanol (80 ml to be added to Washing buffer prior to use) Gloves Isopropanol (100 µl per reaction) Standard table top centrifuge (8000 & 13,000 rpm)	PBS (catalog # 100961) **For yeast:** Low speed centrifuge (3000 x g) and tubes PBS (catalog #100961) Lyticase (catalog #1372467)	Scalpel (optional) **For bacteria:** Low speed centrifuge (3000 x g) and tubes PBS (catalog #100961) Lysozyme (catalog #107255)

Storage Conditions	Shipping Conditions	Product Manager	Available
Room Temperature	Room Temperature	Sharon Spitz x7430	April 15, 1996

Competition

- Our main competitor is Qiagen. When comparing the High Pure PCR Template Prep Kit to the Qiagen kits, the results are:
  - Same or better quality of DNA after isolation
  - This kit can be used for all of the following samples (whole blood, cultured cells, tissue specimens, bacteria and yeast) whereas Qiagen has sample-specific kits
  - Similar or reduced hands-on time requirements (depending on the sample)

  BMB's major advantage over Qiagen is that we offer **complete solutions** (product packages) for premium PCR from start to finish. We offer products used before and after the isolation or purification of nucleic acids (i.e. pre-PCR, PCR, post-PCR products).
- Qiagen's strengths lie in their diverse product line for nucleic acid isolation and purification and that they are the current market leader.
- Qiagen's weaknesses are that their product line is limited to one piece of the PCR puzzle, they are generally high priced, and they rarely discount.
- Promega has one kit that is similar to our kit although Promega is not our main target. See **Extra Information** below for details on Promega's kit.

Supplier	Supplier's Product		Price	Price/rxn	
BMB	**High Pure PCR Template Preparation Kit**	**100 rxns**	**$140.00**	**1.40**	**Blood, tissue, cultured cells, bacteria & yeast**
Qiagen*	QIAamp Blood Kit	50 rxns	$75.00	$1.50	Blood, cultured cells
		250 rxns	325.00	1.30	
	QIAamp Tissue Kit	50 rxns	82.00	1.64	Blood, tissue, cultured cells, bacteria & yeast
		250 rxns	355.00	1.42	
Promega*	Wizard™ Genomic DNA Purification Kit	100 rxns	$130.00	$1.30	Blood, mouse tails, liver, brain, cultured cells, bacteria, yeast
	Wizard™ Genomic DNA Purification Kit (large scale)	50 rxns	300.00	6.00	
Stratagene	DNA Extraction Kit	30-35 rxn‡	$163.00	$4.66-5.43	Blood, cultured cells, tissue

* Details about the Qiagen kits can be found under **Extra Information**.
‡ 35 rxns for blood (10 mL), 35 rxns for cultured cells (1 x 10^8), 30 rxns for tissue (250 mg)

OBF's

Objectives	Benefits	Features
Obtain pure and ready-to-use nucleic acids in minutes from a variety of samples	▪ High purity for reliable and reproducible results ▪ One kit for all purposes	▪ Combination of pre-treatment and highly specific glass fiber fleece adsorption ▪ Function-tested procedure with minimal hands-on time ▪ Technology allows variety of starting materials to be isolated with one product
You can get pure DNA suitable for either PCR or RE digest from your ~~sample quickly~~ and reproducibly due to our combination of sample pre-treatment and our highly specific glass fiber fleece.		
Convenient design	▪ Easy-to-perform protocol with high degree of flexibility	▪ One kit for a large variety of samples
You can achieve rapid (~35 to 95 minutes) and safe ~~DNA extractions from a~~ variety of sample sources with a single kit. The procedure is safe because there are no organics and you can extract you DNA from human blood, cultured cells, tissue, yeast and bacteria.		

Extra Information

Probing Questions :

- What is the source of your DNA (cells, blood etc.) and how are you currently extracting your DNA?
- How would it affect the efficiency of your lab if you needed to purchase just one product for all your DNA extractions and consequently used just one procedure?

Anticipated Customer Questions and Answers:

Q *What is the maximum size sample that can be loaded onto the column?*
A The size of the sample depends on the tissue used . The recommended tissue amounts are 200 µl whole blood , 1.0 x 10^6 cultured cells or 50 mg of tissue. Volume-wise, the maximum amount that can be loaded on the column is 700 µl

Q *Can this kit be used for viral RNA isolation?*
A RNA is co-eluted from your sample, yes, but we have better kits for RNA isolation (mRNA Isolation Kit-general RNA isolation or mRNA Capture Kit-mRNA isolation prior to RT-PCR)

Q *Does the DNA purified with your kit have any RNA in it?*
A Yes, some RNA will co-purify but you can use some RNase, DNase-free if this is a concern for you.

Q *What kind of yield can I expect from my sample?*
A Typically your sample would yield...

Sample type	Amount	Yield in µg
Whole human blood	200 µl	3-6
Cultured K562 cells	1 x 10^6	10
Calf thymus	25 mg	5-10
Bacteria	1 x 10^9	1-3
Yeast	1 x 10^8	10-13

Details of Product Contents:

1. **Tissue lysis buffer**, white cap (20 ml)-4 M urea, 200 mM Tris, 100 mM NaCl, 200 mM EDTA, pH 7.4
2. **Binding buffer**, green cap (20 ml)-6 M Guanadine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton® X-100 (v/v), pH 4.4
3. **Proteinase K**, lyophilizate (90 mg)-90 mg lyophilized. Dissolve in 4.5 ml Di H_2O, aliquot and store at -20°C.
4. **Washing buffer**, blue cap (20 ml)-20 mM NaCl and 2 mM Tris-HCl, pH 7.5 (concentration **after** adding the required 80 ml ethanol)
   NOTE: Customer must add 80 ml of ethanol before using this reagent!
5. **Elution buffer**, colorless cap (40 ml)-10 mM Tris, pH 8.5 (at 25°C)
   NOTE: Elution buffer must be pre-warmed to 70°C in order to work effectively
6. **High Pure filter tubes**-2 bags, each with 50 tubes-Polypropylene tubes with two layers of glass fiber fleece. Capacity is 700µl max.
7. **Collection tubes**-2 bags, each with 50 2 ml polypropylene tubes

Qiagen Product Details:

QIAamp Blood Kit
The purification can be done from up to 200 µl sample volume in about the same hands-on time as for our kit. Larger volume can be processed by loading the QIAamp spin column in multiple steps. The purified DNA is eluted in Tris buffer or water. Depending on sample type, yields of up to 50 µg of DNA can be obtained. The DNA can be isolated from cultured cells, fresh, frozen or dried whole blood (with any anticoagulant), buffy coat, bone marrow, mucus, other body fluids or cell suspension. Viral DNA and RNA can be prepared from plasma or serum. According to their literature, a whole blood sample of 200 µl yields up to 8µg genomic DNA. The isolated DNA ranges up to 50 kb.

QIAamp Tissue Kit
DNA from most human and animal tissue, such as muscle, liver, heart, brain, tumors, rodent tails , blood and body fluids can be purified as well as from cultured cells. Tissue samples require an additional 1-2 hours for lysis; up to 25 mg can be processed in under 3 hours (hands-on time 20 min.). Special protocols for paraffin-embedded tissue, yeast and some bacterial species are available upon request.

Promega Product Details:

Wizard™ Genomic DNA Purification Kit
DNA can be isolated from whole blood, cultured cells, yeast, bacteria, mouse tails, liver and brain tissue using Promega's Wizard™ Genomic DNA Purification Kit. Promega has two pack sizes The 100 rxn size uses sample sizes comparable to BMB's kit (i.e. 300 µl whole blood) whereas the 50 rxn size is for larger scale preps (i.e. 3 mL of whole blood). The procedure for the Wizard™ kit is very similar to ours, including the need to pre-treat yeast and bacteria with a protease. Yields for the Wizard™ kit are comparable to High Pure yields (taking sample size into consideration).

Projected First-Year Sales Impact: $56,000 Total US

Specific Sales Force Instructions: Leverage your customer's satisfaction with our thermostable polymeases to gain business from Qiagen.

Pricing Rational: Penetration pricing strategy-our product will cost considerably less than Qiagen at large institutions and SOS programs where discounts are in place.

Other: Does not appear in 1996 Catalog

Biochemica New Product Entry: April 1996, Number 2, page 4

High Pure PCR Template Preparation Kit
Try our new product for the isolation of nucleic acids for PCR and Southern blotting
Available during Quarter 2, 1996

One crucial factor for the success of a PCR or a Southern blot is the purity of the nucleic acid to be investigated. There are several methods to obtain high purity nucleic acids (e.g., self-made, commercial kits). In all cases the sample (blood, tissue, bacteria, etc.) needs to be checked before an appropriate method is chosen.

Boehringer Mannheim's new High Pure PCR Template Preparation Kit can be used for the isolation and purification of nucleic acids from different sample materials, including whole blood, cultured cells, tissue, bacteria, and yeast. Bacteria and yeast require a specific pre-lysis treatment with lysozyme or lyticase. The DNA can then be used for PCR and/or Southern blots.

Benefits:

- Pure and PCR-ready nucleic acid obtained in minutes
- High purity for reliable and reproducible results
- Reduction of health hazards, no organic compounds are used
- Easy-to-perform protocol with high degree of flexibility.

The principle of the test is simple: after lysis of the tissue sample in lysis buffer, the lysate is applied to a filter tube. The sample is then passaged through a glass filter fleece by centrifugation. Residual impurities are removed by a washing step and, subsequently, nucleic acids are eluted in elution buffer. The nucleic acids purified by the kit can be applied in PCR or restriction endonuclease digestion directly after collection.

* This product is sold under licensing arrangements with Roche Molecular Systems and The Perkin-Elmer Corporation. Purchase of this product is accompanied by a license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with an Authorized Thermal Cycler.

Flow diagram on next page was created without header and footer (does not say "CONFIDENTIAL" etc.) This will allow you to copy it for customers if you wish.

Copy of Sell Sheet and Pack Insert-attached

Flow Diagrams by Sample Type:

Whole Blood or Cultured Cells

Procedure	Time (min.)
Add binding buffer & Proteinase K to sample	5
↓	
Mix & incubate at 72°C	10
↓	
Mix with isopropanol	2
↓	
Pipet sample into filter tube	1
↓	
Centrifuge @ 8000 rpm	1
↓	
Discard flowthrough	1
↓	
Add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Again add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Centrifuge for 10 sec. At 13,000 rpm	1
↓	
Elute DNA	5
↓	
Use immediately or store at 4°C	**Total <35**
Hands-on time <20 minutes	

Tissue

Procedure	Time (min.)
Add Tissue Lysis buffer & Proteinase K to sample	5
↓	
Incubate @ 55°C	60
↓	
Add Binding Buffer	1
↓	
Mix & incubate at 72°C	10
↓	
Mix with isopropanol	2
↓	
Remove insoluble tissue	2
↓	
Pipet sample into filter tube	1
↓	
Centrifuge @ 8000 rpm	1
↓	
Discard flowthrough	1
↓	
Add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Again add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Centrifuge for 10 sec. At 13,000 rpm	1
↓	
Elute DNA	5
↓	
Use immediately or store at 4°C	**Total <95**
Hands-on time <25 minutes	

Bacteria and Yeast

Procedure	Time (min.)
Pellet sample by centrifugation	10
↓	
Resuspend pellet in PBS	3
↓	
Add lysozyme (bacteria) or lyticase (yeast)	2
↓	
Incubate: Bacteria-15 min. @ 37° Yeast-30 min. @ 30°	15-30
↓	
Add binding buffer & Proteinase K to sample	5
↓	
Mix & incubate at 72°C	10
↓	
Mix with isopropanol	2
↓	
Pipet sample into filter tube	1
↓	
Centrifuge @ 8000 rpm	1
↓	
Discard flowthrough	1
↓	
Add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Again add 500 µl wash buffer & centrifuge 1 min. @ 8000 rpm	3
↓	
Centrifuge for 10 sec. At 13,000 rpm	1
↓	
Elute DNA	5
↓	
Use immediately or store at 4°C	**Total <65-80**
Hands-on time <35 minutes	

PURIFICATION

High Pure PCR Template Preparation Kit

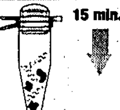
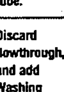
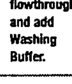
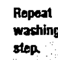
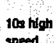
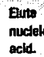
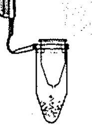
For the isolation of DNA from Human Whole Blood, Cultured Cells, Tissues, Bacteria, and Yeast

Advantages:

- Obtain highly purified DNA in minutes.
- Completely remove contaminants and inhibitors so that DNA templates perform reliably and reproducibly.
- Purify DNA from a wide variety of starting materials with just one kit.

Procedure

(using human whole blood as an example)

Step		Time
Mix sample with Binding Buffer, add proteinase K, and incubate.		15 min.
Mix with isopropanol, and transfer to filter tube.		4 min.
Discard flowthrough, and add Washing Buffer.		4 min.
Repeat washing step.		3 min.
10s high speed.		
Elute nucleic acid.	 	5 min.

Use isolated nucleic acid directly or store at +4°C for later analysis.

TOTAL TIME: <35 min. (hands-on time: <20 min.)

Principle:

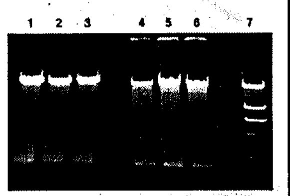
DNA is purified from a wide variety of biological materials by specific binding to and elution from a glass filter fleece contained in a convenient spin filter tube. Sample lysis procedures vary depending upon the biological material used; blood, cultured cells, and tissues require incubation in a special lysis buffer, while bacteria and yeast are treated with either lysozyme or lyticase. Following treatment of all sample types with isopropanol, the samples are pipetted into a filter tube and centrifuged through the glass filter fleece. Residual impurities are removed with Washing Buffer, and the purified DNA is obtained using the Elution Buffer. The highly purified DNA can be used directly for PCR, Southern blots, or restriction enzyme digests.

	300 µl	200 µl	100 µl	Control
MWM	3 µl (4.5 µl)	4 µl (4 µl)	5 µl (2.5 µl)	50 ng
MWM	10 µl (2.5 µl)	20 µl (1 µl)	20 µl (0.1 µl)	10 ng
	50 µl	10 µl	1 µl	Control

Isolation and amplification of a 15 kb tissue plasminogen activator (tPA) fragment from human whole blood.

DNA was isolated from whole human blood according to the protocol given in the High Pure PCR Template Preparation Kit package insert. Blood sample volumes used in the experiment ranged from 1-300 µl. DNA sample volumes (eluate) used after purification ranged from 3-20 µl. The figures in parentheses indicate the sample volume used after taking into consideration the dilution factor.

PCR was performed with the Expand™ Long Template PCR System and human tPA primers specific for a 15 kb fragment.



Comparison of High Pure PCR Template Purification Kit and a leading supplier's product for isolation of genomic DNA.

DNA was isolated from 1 x 10^6 K562 cells according to each product's package insert and evaluated using 8% Agarose MP.

Three separate isolations of genomic DNA from human K562 cells were performed with each kit.

Lanes 1 - 3: Boehringer Mannheim High Pure PCR Template Preparation Kit
Lanes 4 - 6: Leading supplier's product
Lane 7: DNA MWM II

Applications:

- Isolation of high-purity DNA suitable for reliable PCR
- Isolation of DNA from human whole blood, cultured cells, tissues, bacteria, and yeast
- Preparation of DNA for applications requiring larger amounts of template DNA, such as Southern blots and restriction enzyme digests

Typical Results:

Starting Material	Sample Size	Yield
Whole blood, human	200 µl*	3-6 µg (DNA)
Cultured cells, (e.g., K562)	1 x 10^6 cells	15-20 µg (DNA)
Calf thymus	25 mg	5-10 µg (DNA)
Bacteria	1 x 10^9 cells	1-3 µg (total nucleic acids)
Yeast	1 x 10^8 cells	10-13 µg (DNA)

* Typical volume is 200 µl; maximum volume is 300 µl.

Ordering Information:

Product	Cat. No.	Size
High Pure PCR Template Preparation Kit	1796 828	100 reactions

Biochemicals

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High Pure PCR Template Preparation Kit

For isolation of nucleic acids for PCR and Southern blotting

Cat. No. 1 796 828
100 reactions

Product description

Principle: As a prerequisite for the analysis of nucleic acids by the polymerase chain reaction (PCR) or Southern blotting the isolation of the analyte from different sample materials is required. Blood, cell or tissue lysis is accomplished by incubation of the sample in a special buffer in the presence of proteinase K. Subsequently nucleic acids bind specifically to the surface of glass fibers in the presence of a chaotropic salt (1). The binding reaction occurs within seconds due to the disruption of the organized structure of water molecules and the interaction with nucleic acids. Thus, adsorption to the glass fiber fleece is favored. Since the binding process is specific for nucleic acids, the bound nucleic acids are purified from salts, proteins and other cellular impurities by a washing step and are eluted in Tris buffer.

After lysis of the tissue sample in tissue lysis buffer, or for blood or cultured cells directly in binding buffer, the lysate is applied to the filter tube and passaged through the glass fiber fleece by centrifugation. Residual impurities are removed by a wash step and subsequently nucleic acids are eluted in elution buffer.

Application

The *High Pure* PCR Template Preparation Kit is designed for the purification of nucleic acids from different sample material like whole blood, cultured cells and tissue samples. Bacteria and yeast require a specific prelysis treatment with lysozyme or lyticase. Nucleic acids can be applied in PCR or restriction endonuclease digestion directly after elution in Tris buffer. Thus the purification procedure is less time consuming compared with alternative methods which require extraction with organic solutions or DNA precipitation.

The kit contains:

1. **Tissue lysis buffer, white cap**
   One vial (1) with 20 ml tissue lysis buffer consisting of 4 M urea, 200 mM Tris, 100 mM NaCl, 200 mM EDTA, pH 7.4 (25°C).
2. **Binding buffer, green cap**
   One vial (2) with 20 ml nucleic acid binding buffer consisting of 6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton® X-100 (v/v), pH 4.4 (25°C).
3. **Proteinase K, lyophilisate**
   One vial (3) with 90 mg lyophilized proteinase K. Dissolve in 4.5 ml bidest. H_2O, aliquot and store at −20°C.
4. **Washing buffer, blue cap, [add 80 ml ethanol p.a. before first use!]**
   One vial (4) with 20 ml washing buffer consisting of 20 mM NaCl and 2 mM Tris-HCl, pH 7.5 (25°C) (final concentration after addition of ethanol).
5. **Elution buffer, colorless cap**
   One vial (5) with 40 ml elution buffer consisting of 10 mM Tris, pH 8.5 (25°C).
6. **2 bags each with 50 *High Pure* filter tubes**
   Polypropylene tubes have two layers of glass fiber fleece and can hold up to 700 µl sample volume.
7. **2 bags each with 50 collection tubes**
   2 ml polypropylene tubes.

Stability and storage:

The kit components are stable and should be stored at room temperature. After dissolution of proteinase K in bidest. H_2O, the solution should be aliquoted and stored at −20°C; the solution is stable for 12 month.

Standard protocol

Please read before using the kit:

1. Dissolve proteinase K (vial 3) in 4.5 ml redist. H_2O, aliquot and store at −20°C.
2. Add 80 ml ethanol p.a. to washing buffer (vial 4).
3. Before starting a purification reaction warm the elution buffer (vial 5) to 70°C.

   The binding buffer (vial 2) contains guanidine-HCl which is an irritant. Wear gloves and follow usual safety precautions when handling.

Isolation of nucleic acids from whole blood or cultured cells

1. Add PBS to the sample material and fill up to 200 µl. For the standard reaction use 200 µl e.g. whole blood. Add 200 µl binding buffer (green cap) and subsequently 40 µl proteinase K. After proteinase K addition **mix immediately** and incubate for 10 min at 72°C. If larger sample volumes will be used – up to 300 µl of whole blood can be used – increase all volumes accordingly.
2. After the incubation mix samples with 100 µl isopropanol.
3. Combine the *High Pure* filter tube and the collection tube and pipette the sample in the upper reservoir.
4. Centrifuge for 1 min at 8000 rpm in a standard table top centrifuge.
5. Discard the flowthrough and again combine the filter tube and the used collection tube.
6. Add 500 µl wash buffer (blue cap) to the upper reservoir and centrifuge as in step 4.
7. Discard the flowthrough and again combine the filter tube and the used collection tube. Add 500 µl washing buffer (blue cap) to the upper reservoir and centrifuge as in step 4. Finally centrifuge for 10 s at max. speed (13 000 rpm) to remove residual wash buffer.
8. Discard the collection tube and insert the filter tube in a clean 1.5 ml reaction tube.
9. For the elution of the nucleic acids use 200 µl of prewarmed (70°C) elution buffer (vial 5). A higher elution volume can be used to increase the elution efficiency (e.g. 2x 200 µl). Add elution buffer to the filter tube and centrifuge for 1 min at 8 000 rpm.
10. The nucleic acids are stable and can be used directly or stored at 4°C for later analysis.

Isolation of nucleic acids from tissue

1. To the tissue sample, e.g. 25 – 50 mg, add 200 µl tissue lysis buffer (white cap) and 40 µl proteinase K, mix and incubate for 1 h at 55°C. The yield of nucleic acids can be increased by cutting the sample with a scalpel in small pieces before incubation.
2. Add 200 µl binding buffer (green cap), mix and incubate for 10 min at 72°C.
3. Mix with 100 µl isopropanol. Use a 1 ml pipette tip and a pipette to draw in part of the sample volume. This treatment blocks the pipette tip with insoluble tissue parts which can be removed easily. Pipette the bulk of the liquid sample in the combined filter tube and follow the above protocol from step 4 on.

Isolation of nucleic acids from bacteria and yeast

1. Collect the sample by low speed centrifugation (3000 x g) for 5 min and resuspend in 200 µl PBS. Add 15 µl lysozyme for bacteria (10 mg/ml in Tris-HCl, pH 8.0) or 10 µl lyticase* for yeast cells (0.5 mg/ml). Incubate for 15 min at 37°C (30 min at 30°C for yeast).
2. Subsequently add 200 µl binding buffer (green cap). Add 40 µl proteinase K, mix immediately and incubate for 10 min at 72°C.
3. Follow protocol as described for blood and cell culture from step 2.

Experimental results

Whole blood human (µl)	Yield (µg)
200	3 – 6

Cultured cells K 562	Yield (µg)
1×10^6	10

Calf thymus (mg)	Yield (µg)
25	5 – 10

Quality control

25 mg calf thymus are treated as described in the protocol for tissue samples. After RNase treatment the DNA concentration is determined. At least 5 µg of DNA is isolated. 100 ng of the nucleic acid is used with specific primer for amplification of a 1.1 kb DNA fragment from the gene for terminal transferase and the expected amplification product is obtained. The quality of the nucleic acid is controlled in an Expand™ Long Template PCR with a 1.1 kb amplification product.

Notice to purchaser

This product is optimized for use in the Polymerase Chain Reaction ("PCR") covered by patents owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd. ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as Boehringer Mannheim when used in conjunction with an authorized thermal cycler, or is available from the Perkin-Elmer Corporation. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at the Perkin-Elmer Corporation, 850 Lincoln Center Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Reference

1 Vogelstein, B., & Gillespie, D (1979) Proc. Natl. Acad. Sci. USA 76,

* available from Boehringer Mannheim GmbH

Triton® is a registered trademark of Rohm & Haas Company, Philadelphia, PA, USA.

DNA Isolation Kit for Mammalian Blood

Catalog #	Pack Size	Price	Priority	Product Class
1667 327	25 rxns (10 mL samples)	$99.00	B *Add-on or opportunistic product/Me-Too product*	18 *Separation Technology*

What Does It Do and How Does It Work?

Add **Red Blood Cell Lysis Buffer** to 10 mL blood sample
⇓
Incubate 10 min. RT on gyratory shaker then centrifuge 10 min.
⇓
Remove supernatant. Vortex pellet in residual buffer then resuspend pellet in **White Blood Cell Lysis Buffer**.
⇓
OPTIONAL-RNase treatment
⇓
Add **Protein Precipitation Solution** and vortex. Centrifuge for 10 min.
⇓
Ethanol precipitate DNA (13 min.)
⇓
Resuspend DNA in TE
⇓
DNA is now ready for PCR or Southern blot

Total Time ≤1.5 hour‡
Hands-On Time ≤15 min.

A variety of methods have been developed to prepare template DNA from human whole blood for amplification. The most frequently used methods employ Ficoll®/hypaque gradients and phenol/chloroform extractions. However, these methods require extensive hands-on time and pose health risks associated with exposure to biohazardous materials. The number of human blood samples prepared for the polymerase chain reaction (PCR) and genomic Southern blots is growing rapidly. Consequently, the need for a faster, easier, and safer method increases.

DNA Isolation Kit for Mammalian Blood rapidly prepares mammalian whole blood, lymphocytes and buffy coat DNA for direct use in all applications, including PCR (standard and long PCR) and Southern blotting. Also, the extractions are performed without the use of hazardous organic solvents or chaotropic agents.

Protocol Synopsis

After mixing the blood sample with **Red Blood Cell Lysis Buffer**, incubating and centrifuging, **White Blood Cell Lysis Buffer** is added to break open the white blood cells. **Protein Precipitation Solution** is added followed by centrifugation to pellet proteins. The last step is ethanol precipitation of the DNA. The now pure genomic DNA is ready for PCR, restriction digestion prior to Southern blotting, DNA sequencing et cetera. The total time is ~90 minutes (not including 30-60 min. final resuspension of DNA) with only about 15 minutes hands-on time (for 4 samples). The progressive layout of the package insert indicates actual time and hands-on time for every step of the procedure (as well as important notes for each step-See attached pack insert). Customers can use fresh blood or blood stored for ≤3 days and expect optimal yields. Blood stored for 7 days at +4°C or ≤1 month at -20°C can also be used but yield will be reduced ~10-15%. See **Extra Information** below for chart of average yields, details of blood storage options, QC using Expand™ LT and schematic of procedure.

Some **companion products** would be: Expand™ High Fidelity, Expand™ Long Template, Expand™ 20kb^{Plus}, *Taq* DNA polymerase, *Pwo* DNA polymerase, Restriction Enzymes, Genius Starter Kit II, Genius System, High Prime DNA Labeling Mix, PCR Nucleotide Mix, PCR Core Kit, dNTP's, Repli-pack Reagent Set, PCR Optimization Kit, Agarose MP, DNA Molecular Weight Markers

‡ Time estimates do not include final resuspension of DNA. Resuspension time varies but can be expected to take 30-60 min.

Summary

- DNA is extracted from **mammalian** whole blood, lymphocytes or buffy coat quickly and easily without using hazardous reagents in ≤90 minutes total time and ≤15 minutes hands-on time..
- The DNA is ready for direct use in standard and long template PCR, sequencing, Southern blots, etc.

Who Should I Talk To?

Clinical and research labs that use mammalian blood as a source of genomic DNA for PCR, sequencing and Southern blotting. This would include customers doing CFLP, RFLP, SSCP, etc. To analyze genetic mutations.

Some buzzwords to tip you off: **human genomic DNA, Southern, RFLP, CFLP, SSCP, sequencing, Human Genome Project, PCR, amplification, blood, lymphocytes, buffy coat, template prep.**

Techniques Associated with Product		Contents (Physical Description)
Replaces: Ficoll®/hypaque gradients Phenol/chloroform extractions Column-based purification (*i.e.* Qiagen)	**Precedes:** PCR Southern blotting (including CFLP, RFLP, SSCP, DGGE) Sequencing	**Red Blood Cell Lysis Buffer**, 750 mL **White Blood Cell Lysis Buffer**, 125 mL **Protein Precipitation Solution**, 65 mL

Additionally Required Materials:	
Mammalian Blood Sample Ethanol 70% Ethanol TE Buffer, pH 8.0 (Tris 10 mM, EDTA 1 mM)-Optional RNase-Optional 50 mL Centrifuge Tubes, sterile-must withstand a minimum of 900 x g, preferably 12,000 x g)-2 to 3 per sample	17 x 100 mm tube, sterile-as necessary, see note for Step 9 (pack insert on page 2). Rocking platform or Gyratory shaker-highly recommended Vortexer Centrifuge(s)-capable of spinning 50 mL tubes at 875 x *g* and 12,000 x *g** (*e.g.* Sorvall RT6000B and Sorvall RC5B, respectively)

*See pack insert for details.

Storage Conditions	Shipping Conditions	Product Manager	Available
Room Temperature	Room Temperature		

Author's Note-Competitor Information will *follow* OBF's for this issue only

OBF's

Objectives	Benefits	Features
Pure DNA for multiple downstream applications	▪ DNA is pure enough to provide excellent PCR, Southern blotting and cloning results ▪ High yield of pure DNA potentially reduces amount of precious sample required and/or experimental repeats	▪ Produces purified DNA-A_{260}/A_{280} ratio typically 1.7-1.9 ▪ QC tested for function-4.8 kb fragment is amplified using Expand™ LT. ▪ DNA can be used for PCR. Southern blots, sequencing, cloning, etc. ▪ DNA yield for human whole blood is avg. of 35 µg/mL of blood
You will get a good yield of DNA, an average of 35 µg of DNA per ml of blood, that has a typical A_{260}/A_{280} ratio of 1.7-1.9. You will be able to use this purified DNA in a number of applications including PCR, sequencing, cloning and Southern blotting which allows you to use one kit for all your blood DNA isolation. You will conserve precious biological samples and have fewer experimental repeats due to "dirty" DNA because our kit gives you high yield and high purity DNA.		
Fast and easy DNA isolation	▪ Faster than "home brew" methods and most other commercial products ▪ Kit is easy to use, fewer unanswered questions and fewer mistakes by user which means first-time success and consistency	▪ Procedure takes ≤15 minutes hands-on (4 samples) and ≤1.5 hours total (plus 30-60 minutes to resuspend DNA). ▪ Progressive layout of pack insert is easy-to-follow and has notes, actual time and hands-on time for every step
The simple DNA extraction procedure takes 1.5 hours with just 15 minutes hands-on time which makes multiple sample processing fast and easy. The straight-forward protocol is outlined in a unique way in the package insert where each step has informative notes, actual time and hands-on time. The layout is such that, despite the addition of the bonus information, the procedure is concise and easy to follow.		
Flexible	▪ DNA can be isolated from a variety of sources and forms with a single kit. ▪ Flexible storage options, starting volumes and pre-treatment options ensures a high yield of purified DNA from a myriad of mammalian blood samples.	▪ Can use whole blood, buffy coats or lymphocytes from a variety of mammals. ▪ Blood used fresh or stored for ≤ 3 days provides 100% of expected yield. Samples stored at 4°C for 7 days or ≤ 1 month at -20°C lose just 10-15% yield. ▪ Pack insert provides protocol for using samples from 1 ml up to the standard 10 ml volumes. ▪ Blood can be treated with sodium heparin, sodium citrate or EDTA prior to procedure
This kit is very flexible. You will be able to get your blood samples from a variety of mammals and from whole blood, lymphocytes or buffy coats. In addition, you have flexibility in the storage, volume, and the chemical treatment of your blood samples during collection.		

Other OBF's: Safety- *You can feel safer while using this kit because you are not using any chaotropic agents or organics.* **Save Money**-*Not only is our kit less expensive than most kits on the market, it also provides procedures for smaller samples. This will reduce the use of reagents stretching your grant dollars further.*

Competition

- The majority of researchers purify DNA from blood using "home brew" methods. Most of these home brew methods use organics like phenol and take several hours to several days to produce purified DNA.
- Qiagen uses a column-based method for isolating DNA from blood. The techniques relies on gravity flow of DNA and cellular debris through the column. The instructions for the Qiagen kit stipulate a specific range of white cell density. If the cell number is too high, the column will be overloaded with DNA and will clog; if the white cell number is to low, special steps must be taken during precipitation (just as with our kit). It is inconvenient for the customer to have both a minimum and maximum number of white cells in a sample for a technique to work. This makes our kit more convenient than Qiagen's. It would be not only inconvenient but also potentially destructive to have a clogged column that may loose precious sample.

Supplier	Supplier's Product		Price	Price/ rxn	mL of blood
BMB	**DNA Isolation Kit for Mammalian Blood**	**25 rxns**	**99.00**	**$3.96**	**10 mL samples**
Qiagen	Qiagen Blood & Cell Culture DNA Kit	25 rxns 10 rxns	188.00 175.00	$7.52 $17.50	1-5 mL samples 5-20 mL samples
Gentra	Puregene™ DNA Isolation Kits	3 rxns 12 rxns 100 rxns	$60.00 $150.00 $895.00	$20.00 $12.50 $9.00	10 mL samples
Oncor	Non-Organic DNA Extraction Kit for Mammalian DNA	20 rxns	$200.00	$10.00	5-10 mL samples

For more information on these and other competitors, see "**Detailed Competitor Comparison**" below.

Extra Information

Probing Questions :

How do you currently extract your DNA from blood?

Anticipated Customer Questions and Answers:

Q *What yield of DNA does the kit provide?*

A The amount of DNA recovered will vary, depending on the number of white cells present in the donor blood. The average yield for healthy human whole blood is 350 µg for a 10 mL sample. The range of yields for a 10 mL sample is from 200 µg up to 700 µg. [See chart below or pack insert for other species]

Q *My blood samples have a lower than normal number of white cells. Will this kit still work?*

A Yes. Follow the procedure as for normal blood but use higher centrifugation and a glycogen carrier in the ethanol precipitation step. The details are outlined in the pack insert.
[**NOTE**: *healthy human blood has an average of 5×10^6 white cells per mL, 5×10^7 white cells for a 10 mL sample. The pack insert suggests using a glycogen carrier and higher centrifugation speeds when a sample contains less than a total of 1.5×10^7 white cells (i.e. about 1/5 the normal amount)*]

Q *My DNA samples are from leukemia patients so they have an elevated white cell count. Will this be a problem?*

A No. Unlike some column-based methods, there is no upper limit on white cell count.

Q *I don't have 10 mL of blood. Can I use less?*
A Yes. The pack insert provides instructions for samples as small as 1 mL.

Q *How long can my samples be stored and at what temperature?*
A For fresh samples or samples stored up to 3 days, you can expect 100% of your expected yield. If you choose to store your samples for as long as 1 month at -20°C or a week in the refrigerator you will still get 85% to 90% of the expected yield.

Q *I get my samples from a clinical lab. What type of tubes (anti-coagulants) can they use to collect my blood?*
A Samples can be treated with heparin, sodium citrate or EDTA which will allow you to use clinical samples without disrupting the clinical labs' standard procedures. [See Pack Insert or **Details of Blood Storage Options** below for details]

Q *Can the kit be stored in the freezer, or at room temperature?*
A The kit is stable for 2 years when stored at room temperature.

Q *The kit prepares DNA in less than 90 minutes. How much of that time is hands-on time?*
A ~15 minutes for 4 samples.

Q *Are any additional reagents required?*
A Yes. Ethanol & 70% Ethanol. TE Buffer and RNase are optional.

Q *What equipment will I need to use the kit?*
A For each sample

1. Vortexer
2. Centrifuge that can accommodate 50 mL tubes and spins at least 900 x g
3. Rocking platform or Gyratory shaker (highly recommended)

If 50 mL Centrifuge Tube can withstand ≥ 12,000 x g:	**If 50 mL Centrifuge Tube can *NOT* withstand ≥ 12,000 x g:**
4. 2 each: 50 mL Centrifuge Tubes, sterile capable of withstanding *at least 12,000 x g*	4. 2 each: 50 mL Centrifuge Tubes, sterile capable of withstanding *at least 900 x g*
5. Centrifuge that can accommodate 50 mL tubes and spins at least 12,000 x g (can be same instrument as 2. above)	5. 1 each: 17 x 100 mm sterile centrifuge tube capable of withstanding at least 12,000 x g
	6. Centrifuge that can accommodate the 17 x 100 mm tubes and spins at least 12,000 x g (can be same instrument as 2. above)

Q *With this method, how many samples can be processed at the same time?*
A The only limit is in the number of samples your centrifuge can handle at one time.

Average Yields (whole blood):

Chart below appears in the package insert on page 3.

Species	Average yields	Yield range
Mouse	570 µg/10 ml blood	430-670 µg
Rat	580 µg/10 ml blood	350-680 µg
Dog	450 µg/10 ml blood	350-600 µg
Porcine	670 µg/10 ml blood	520-780 µg
Guinea Pig	160 µg/10 ml blood	55-295 µg
Human*	350 µg/10 ml blood	200-700 µg

* The average yield and yield range for healthy human blood is not shown in the package insert chart but is stated in the paragraph just above the chart.

Other blood forms as starting material:

Source	Average yield	# of white cells
Lymphocytes isolated from whole blood prior to procedure (i.e. using Ficoll®-Hypaque):	75-300 µg	$1.1\text{-}4.2 \times 10^7$
Buffy Coat	35-105 µg	$1.1\text{-}2.3 \times 10^7$

Details of Blood Storage Options:

Customers should use sodium heparin, sodium citrate, or EDTA as anti-coagulants. NOTE: when heparin is used, the white cell pellet in Step 7 must be heated for 10 min. at 65° in the White Blood Cell Lysis Buffer. This is clearly noted in the pack insert on page 1, column 2 under "Starting material:" and as a note for Step 7 on page 2.

Quality Control:

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. All kit components are tested for the absence of DNases (see pack insert for details).

Projected First-Year Sales Impact:

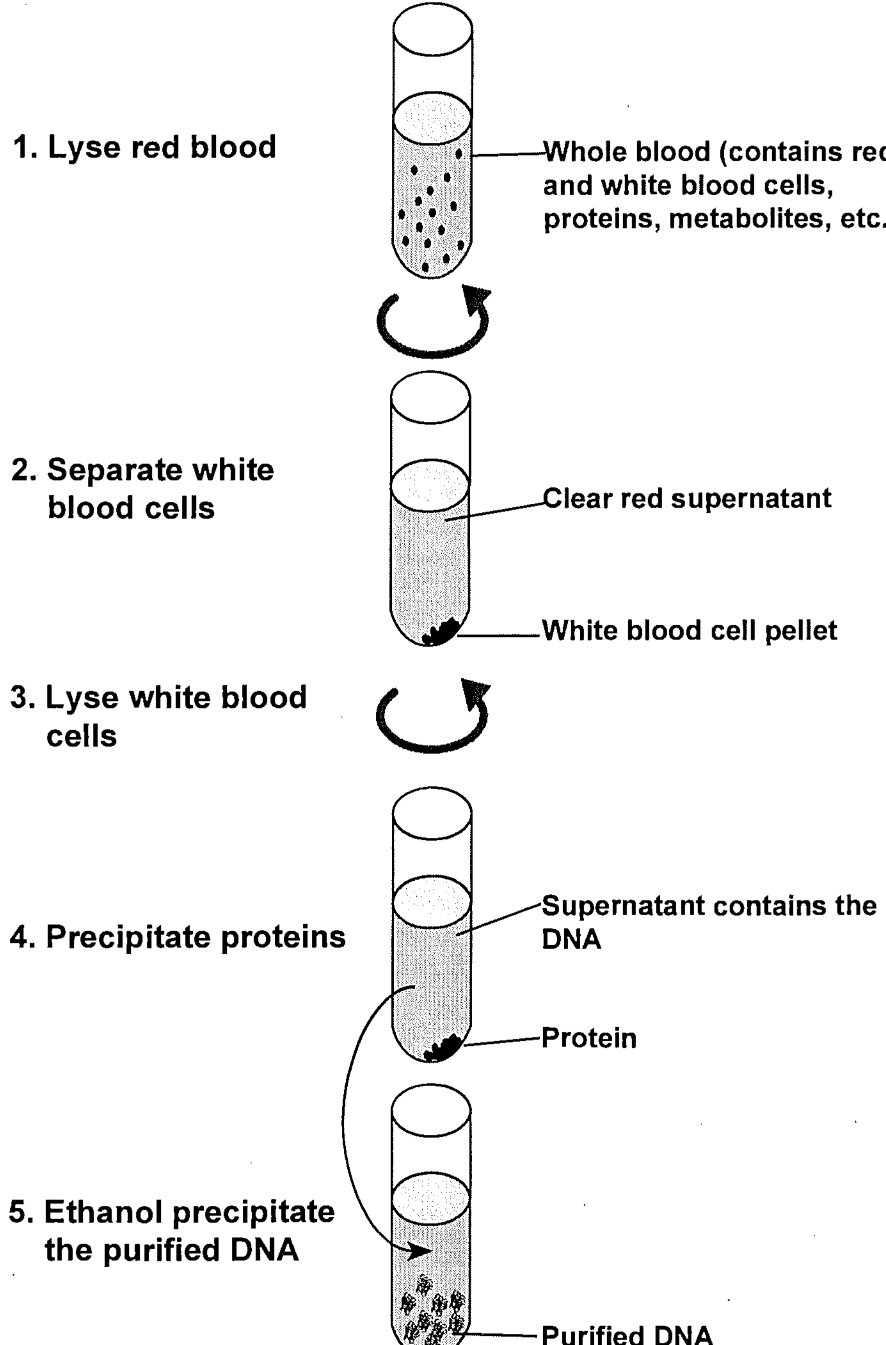
$99,585 for entire US

Pricing Rational: Competitive with home-brew methods and Qiagen.

New Product News: Issue Number 4 (blue-   )-See attached copy of article.

Biochemica:

Detailed Schematic of Procedure



Detailed Competitor Comparison

Company	Product Name	Hazardous Materials/ Proteases	EtOH ppt Req'd	Total Procedure Time	Starting Material	Downstream Application	DNA Yield	Sample Volume	Pack Size	Cost	Cost per Assay	Cost/ ml of blood
BMB	**DNA Isolation Kit for Mammalian Blood**	**No organics; requires Pro K**	**Yes**	**≤1.5 h + rehydration (30-60 min. @ 65°C)**	**Mammalian whole blood, buffy coat, lymphocytes**	**Southerns, RFLP, PCR, Sequencing**	**20-70 µg/ml blood**	**10 ml**	**25 rxn**	**99.00**	**3.96**	**$0.40**
Qiagen	Qiagen Blood & Cell Culture DNA Kit Midi Kit Maxi Kit	No organics; requires Pro K	Yes	<3 h	Whole Blood, Buffy Coat, Cultured cells	Southerns, RFLP, PCR	15-20 µg/ml blood	**midi**: 5 ml **maxi**: 20 ml	midi: 25 maxi: 10	$180.00 $170.00	$7.20 $17.00	$1.44 $0.85
Gentra	Puregene™ DNA Isolation Kits	No organics; requires RNase	Yes	1.5 h + ON rehydration or 1 h at 65°C	Whole Blood, Bone marrow	Southerns, PCR, cloning	35 µg/ml blood	variable up to 12 ml max.	10 ml blood: 3 rxns 12 rxns 100 rxns	 $60.00 $150.00 $895.00	 $20.00 $12.50 $9.00	 $2.00 $1.25 $0.90
Oncor	Non-Organic DNA Extraction Kit for Mammalian DNA	No organics	Yes	3 h + ON rehydration	Buffy coat, separated lymphocytes, body fluids, cultured cells, tissue	RE Digests, Southerns	75-140 µg per 2.5×10^7 cells	5-10 ml	20 rxns	$200.00	$10.00	$1.00-$2.00
Invitrogen	Turbogen™ Kit	Organic Phase-Sep column	Yes	<1 h	Tissue, Cultured cells, Blood	PCR, RE mapping, Southerns, RFLP, cloning	20-80 µg per 10^7 cells	2 ml	25 rxns	$195.00	$7.80	$3.90
Pharmacia	RapidPrep™ Macro Genomic DNA Isolation Kit for Blood	No organics or proteases; uses chaotrope	Yes	1.5 h	Mammalian Blood (Human)	PCR, RFLP, etc.	30-40 µg/ ml blood	1-5 ml blood	10 rxns	$145.00	$14.50	$2.90-$14.50
Scotlab Inc.	Nucleon II	Chloroform, RNase A	Yes	<2 h	Whole Blood, Cultured Cells	PCR, RFLP	22.6 µg/ ml blood	5-10 ml	≤50 rxns (5-10 ml blood)	$155.00	$3.10	$0.31-$0.62
AGTC	SuperQUIK-GENE	No	Yes	<2 h	Whole Blood	RFLP, PCR	25-30 µg/ ml blood	≤8.5 ml can scale	100 rxn 10 rxn	$150.00 $20.00	$1.50 $2.00	$0.18-$0.23

(shaded) = Top competitor

Company	Product Name	Hazardous Materials/ Proteases	EtOH ppt Req'd	Total Procedure Time	Starting Material	Downstream Application	DNA Yield	Sample Volume	Pack Size	Cost	Cost per Assay	Cost/ ml of blood
Stratagene	DNA Extraction Kit	No organics, RNase, Pronase	Yes	2 h blood	Whole Blood, Cultured Cells, Solid Tissue	RE digests, cloning, Southerns, PCR, etc.	>10 µg/ ml blood	5-10 ml	35 rxns	$163.00	$4.66	$0.93 $0.47
Washington Biotechnology Inc	Genomix Scale-Up	Requires Chloroform	Yes	≤ 1 h	Human Blood, White Cell Nuclei	RE digests, Southerns, PCR, etc.	30-60 µg/ ml blood	10 ml	60 rxns	$240.00	$4.00	$4.00
Oncogene Science	Quick Clean™ DNA Extraction System	No organics, RNase, Pronase	Yes	2-4 h	Blood, Cultured Cells, Solid Tissue	Re digests, cloning, Southerns, PCR, etc.	--	10 ml	35 rxns	$140.00	$4.00	$0.40
Life Technologies	Genomic DNA Isolation System	No organics, Pro K	Yes	5.5 h + ON DNA resuspension	Blood, Cultured Cells	Re digests, cloning, Southerns	--	5 ml	30 rxns	$159.00	$5.30	$1.06
Biotecx Laboratories	"DNA Single Tube"	Phenol, Chloroform	Yes	≥2 h + 1 h to ON resuspension	Blood, Cultured cells, Tissue	PCR, Southerns	~43 µg/ ml blood	7 ml	25 rxns	$170.00	$6.80	$0.97
Cruachem Ltd	Isolate 2 Kit	--	--	--	Whole Blood	--	~25 µg/ ml blood	10 ml	50 rxns	$150.00	$10.00	$3.00
BIO 101	G/NOME DNA Whole Blood Isolation Kit	No Organics	Yes	--	Whole Blood	RE digests, PCR	6.5-15 µg/ ml blood	4 ml	10 rxns 25 rxns 100 rxns	$40.00 $85.00 $265.00	$4.00 $3.40 $2.65	$1.00
"Home-Brew"	Single Reagents	Organics , Pro K used	Yes	>3 h	--	PCR, Southerns, Expand		10 ml average	--	--	$1.95- $3.85	$0.20 $0.39

- Triple Helix™ Plasmid Insert DNA Isolation Kit
- DNA Isolation Kit for Mammalian Blood
- Alkaline Phosphatase, Shrimp
- DNA Packaging Kit

DNA Isolation Kit for Mammalian Blood: Economical, easy-to-use DNA purification method

Boehringer Mannheim is proud to introduce a new DNA purification kit for the isolation of genomic DNA from mammalian whole blood, lymphocytes, and buffy coat. The **DNA Isolation Kit for Mammalian Blood** provides all the necessary reagents for the rapid isolation of purified genomic DNA, free of contaminating heme and proteins. The resulting DNA is pure, has a high molecular weight, and is ready for use in all applications—including genomic Southern blots (Figure 1) and standard and long template PCR.

The isolation method (Figure 2) involves the sequential lysis of red blood cells followed by white blood cell lysis. Proteins are removed using a protein precipitation step. Ethanol precipitation and subsequent resuspension of the DNA results in purified DNA that is ready to use in all applications.

With this kit you can

- **Process multiple samples simultaneously** – Because the method is so simple—involving a minimal number of hands-on steps and no column purification or organic extractions—multiple samples can easily be processed simultaneously.

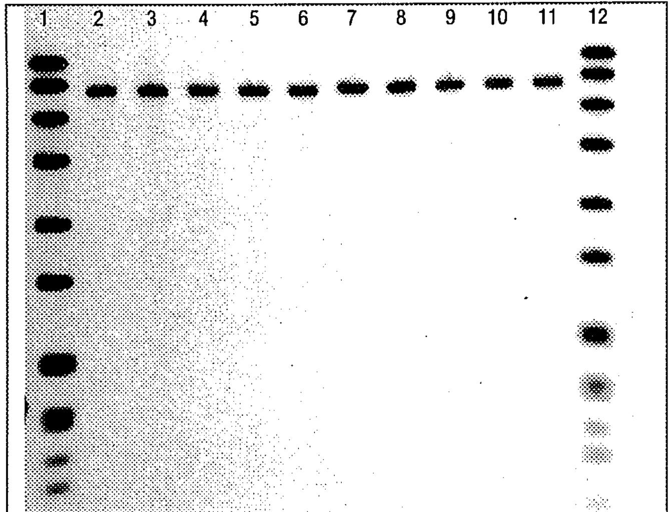


Figure 1. *N-ras* genomic Southern blot using human DNA isolated with the DNA Isolation Kit for Mammalian Blood. DNA was prepared from 10 ml of human whole blood (stored in various anticoagulants), from buffy coat, and from lymphocytes using the DNA Isolation Kit for Mammalian Blood. The isolated DNA samples, 10 µg per lane, were digested with *Eco* RI, electrophoresed, and transferred to nylon membranes. The transferred DNA was then hybridized with a 1.5 kb DIG-labeled *N-ras* probe (15 ng/ml). The membrane was subsequently washed, blocked, and immunodetected with the DIG/Genius™ Nonradioactive System and CDP-*Star*™ Chemiluminescent Substrate. The Southern blot was exposed to Lumi-Film Chemiluminescent Detection Film for one minute.

Lanes 1,12: DNA Molecular Weight Marker VII, digoxigenin-labeled; Lanes 2,3: human whole blood; sodium citrate anticoagulant; Lanes 4,5: human whole blood; sodium heparin anticoagulant; Lanes 6,7: human whole blood; EDTA anticoagulant; Lanes 8,9: buffy coat; Lanes 10,11: lymphocytes

- **Easily prepare pure DNA in less than 90 minutes** – No waiting for columns to drip and no multiple wash steps or tedious Ficoll® Hypaque so you can prepare pure DNA ($OD_{260/280}$ = 1.8–1.9) in only 90 minutes (additional time necessary for resuspension).
- **Purify DNA using the convenience of a kit, but for the cost of most homemade methods** – This kit's price is comparable to home-brew methods, yet it provides the convenience of ready-to-use and function-tested solutions and detailed procedures.
- **Enjoy consistent and reliable results** – Not only is each component of the kit tested to be DNase free, but DNA isolated with the kit is also tested using the Expand™ Long Template PCR System to ensure the functionality of your isolated DNA.

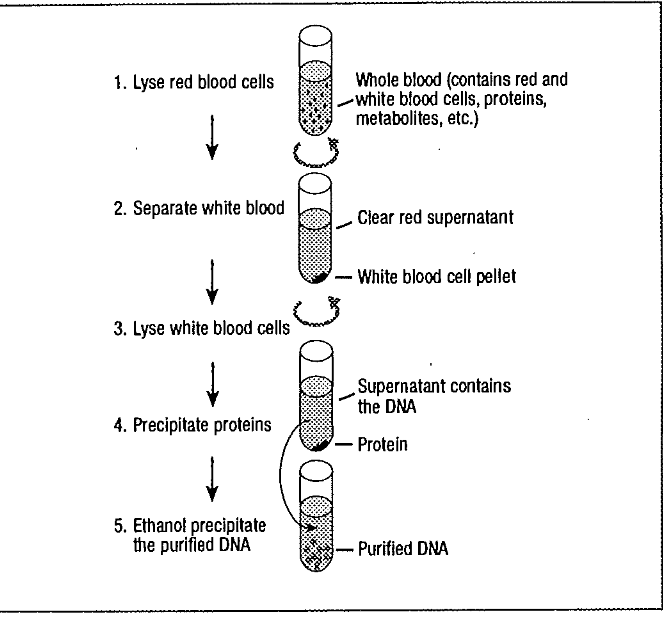


Figure 2. Schematic of the DNA Isolation Kit for Mammalian Blood procedure.

Product	Cat. No.	Size	Price
DNA Isolation Kit for Mammalian Blood	1667 327	1 kit	$99.00
Contents (For 25 reactions, 10 ml blood per reaction):			
1. Red Blood Cell Lysis Buffer (750 ml)			
2. White Blood Cell Lysis Buffer (125 ml)			
3. Protein Precipitation Solution (65 ml)			

CDP-*Star* is a trademark of Tropix, Inc.
Expand is a trademark of Boehringer Mannheim.
Ficoll is a registered trademark of Pharmacia Biotech AB.
Genius is a tradename used in the U.S.

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

DNA Isolation Kit for Mammalian Blood

Cat. No. 1 667 327

Size: up to 10 ml mammalian blood

Product Description

Intended use: The DNA Isolation Kit for Mammalian Blood simplifies the rapid isolation of DNA from 1–10 ml whole blood (*e.g.*, from human, mouse, rat). The isolated DNA is suitable for many applications, including standard PCR, long PCR, sequencing, and Southern blots. In addition to the standard "DNA isolation from 10 ml mammalian whole blood" procedure, this kit permits the isolation of DNA from buffy coat and lymphocyte samples.

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, or red blood cells, which lack nuclei, and therefore, possess no DNA. Only leukocytes (0.3% of total blood cells), or 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 (1).

The DNA isolated from whole blood must be of free of contaminants that may interfere with such applications as PCR. For example, the presence of contaminating heme in the final DNA preparation has been shown to strongly inhibit *Taq* DNA polymerase during PCR (2).

The DNA Isolation Kit for Mammalian Blood provides a simple, rapid, and safe method for the effective isolation of pure DNA. The entire procedure can be completed in less than 1.5 h (plus the resuspension time), and this easy-to-use kit employs fewer steps than standard methods (*e.g.*, Ficoll-Hypaque® density gradients). This simplifies the simultaneous processing of multiple samples. Safety is another key attribute of the kit, which reduces handling of blood and eliminates the need for organic extractions or chaotropic agents.

Principle: 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 (3,4).

Kit contents:

1. Red Blood Cell Lysis Buffer, 750 ml
2. White Blood Cell Lysis Buffer, 125 ml
3. Protein Precipitation Solution, 65 ml

Stability and storage: The kit components are stable through the control date printed on the box (24 months from date of manufacture) when stored at room temperature.

Applications

Safety precautions: Employ universal safety precautions when working with biohazardous materials. Wear lab coats, gloves, and safety glasses at all times. Properly dispose of all contaminated materials. Decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

Additionally required materials: In addition to the kit components, these additional materials will be needed for DNA isolation from mammalian blood:

- Ethanol
- 70% Ethanol
- TE Buffer, pH 8.0 (optional)
- RNase (optional)

DNA Isolation from 10 ml mammalian whole blood:

Starting material:

- 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 4°C or –20°C, warm the blood to room temperature 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 $\leq$3 days. Blood stored for 7 days at 4°C or $\leq$1 month at –20°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 min at 65° to facilitate lysis (step 7).

Procedure: Perform all steps at room temperature unless otherwise indicated.

Step	Isolation Procedure	Notes	(for 4 samples) Actual time	Hands-on time
1	For each blood sample to be processed, 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 x *g* (preferably 12,000 x g) and accommodate a total volume of 40 ml.	30 sec	30 sec
2	To each tube, add 10 ml mammalian blood. Cap the tube, and mix gently by inversion.	Do not vortex!	30 sec	30 sec
3	Place the centrifuge tube on a rocking platform or gyratory shaker for 10 min.	Alternatively, manually invert the sample periodically for 10 minutes.	10 min	30 sec
4	Centrifuge the tube at 875 x *g* for 10 min (*e.g.*, 2,000 rpm in a RT6000B Sorvall Centrifuge).	Do not exceed centrifugation speed limit, as this will increase the difficulty of resuspending the white cell pellet (step 6).	12 min	1 min

Step	Isolation Procedure	Notes	(for 4 samples) Actual time	Hands-on time
5	Carefully pour off and properly dispose of the clear, red supernatant (indicative of complete 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 room temperature before repeating steps 1–4.	30 sec	30 sec
6	Thoroughly vortex the white pellet visible at the bottom of the tube in the residual supernatant.	• *Vortex thoroughly*, making sure that the white cell pellet is fully resuspended in the residual supernatant. This step facilitates complete lysis of the white cell pellet during step 7. • Note that the white cell pellet will still be slightly red in color due to the presence of residual hemoglobin. The hemoglobin will be removed in subsequent steps.	1 min	1 min
7	Add 5 ml White Cell Lysis Buffer, cap the tube, and mix thoroughly by vortexing.	• *Vortex thoroughly* to ensure that 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 minutes, which may facilitate lysis. • For heparin-treated blood, heat the white cell pellet in White Blood Cell Lysis Buffer for 10 min at 65°C to facilitate lysis.	1 min	1 min
8	**Optional:** Add RNase* to a final concentration of 0.02 µg/µl. Mix by inversion, and incubate at 37°C for 15 minutes.	• RNase treatment completely removes RNA contaminants from the final DNA sample, yielding pure DNA with no RNA contamination. However, if subsequent applications do not require a RNA-free sample, this step can be eliminated. • The incubation time can be extended up to 1 h at 37°C.	15.5 min (optional)	30 sec
9	Transfer the sample to a sterile 17 x 100 mm tube.	The sample can remain in the 50 ml centrifugation tube if the 50 ml tube is capable of withstanding 12,000 x *g* centrifugal force.	30 sec	30 sec
10	Add 2.6 ml Protein Precipitation Solution to each sample. Vortex thoroughly.	• *Vortex thoroughly!* (We recommend that you vortex continuously for approximately 25 seconds.) This is necessary for effective removal of protein from the sample. • Upon vortexing, a brownish protein precipitate will be clearly visible.	1 min	1 min
11	Centrifuge the sample at 12,000 x *g* (*e.g.*, 10,000 rpm in a Sorvall RC5B centrifuge) for 10 minutes.	Samples must be centrifuged at 12,000 x *g* for a minimum of 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 min	1 min
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 accommodate approximately 30 ml and withstand a minimum of 900 x *g* (or 12,000 x *g* for isolation of <1.5×10^7 cells).	30 sec	30 sec
13	Ethanol precipitate the DNA: **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.	• Add 2 volumes of room temperature 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 x *g* for 10 minutes (*e.g.*, 2,000 rpm in a Sorvall RT6000B centrifuge). Discard the supernatant. For <1.5×10^7 cells, centrifuge at 12,000 x *g* for 10 minutes following addition of glycogen carrier. • 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 x *g* to facilitate effective precipitation.	13 min	1 min

Step	Isolation Procedure	Notes	(for 4 samples) Actual time	Hands-on time
14	Add 3 ml cold 70% ethanol to the DNA pellet in the tube, and carefully mix the sample several times by gentle inversion. Centrifuge the sample at 875 x *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 $<1.5 \times 10^7$ cells, centrifuge the DNA at 12,000 x *g* for 5 minutes after the 70% ethanol wash.	8 min	1 min
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 over-dry the DNA pellet, as this will make it much more difficult to fully resuspend the DNA.	5 – 15 min	0–1 min
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 30–60 min to aid in resuspension; periodically vortex the samples.	• *Vortex thoroughly* to help resuspend the DNA pellet. • For human blood samples, a 30-minute incubation at 65°C is sufficient to fully resuspend DNA samples. For samples from other mammalian species, place the samples at 65°C for 60 minutes.	1 min + Resuspension (30 – 60 min)	1 min
17	Store samples at 4°C until use.	If desired, samples can be accurately quantified using spectrophotometry or fluorometry.		

Total time: ≤1.5 h + Resuspension (for 4 samples)
Hands-on time: ≤15 min

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.

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 obtained from other species:

Species	Average yields	Yield range
Mouse	570 µg/10 ml blood	430–670 µg
Rat	580 µg/10 ml blood	350–680 µg
Dog	450 µg/10 ml blood	350–600 µg
Porcine	670 µg/10 ml blood	520–780 µg
Guinea Pig	160 µg/10 ml blood	55–295 µg

The A_{260}/A_{280} ratio for isolated DNA samples is typically 1.7–1.9.

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 Lysis Buffer Volume (ml): (step 7)	Protein Precipitation Solution Volume (ml) (step 10)	Recommended Resuspension Volume (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

Adjust the size of the centrifuge tube as necessary to accommodate the volumes being used.

Procedural modifications for use with buffy coat or lymphocyte samples: Freshly isolated buffy coat or lymphocyte samples 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:

a. Isolate the lymphocyte population from 10 ml human blood, following the procedure outlined in the package insert provided with the lymphocyte separation medium you choose (e.g., using standard Ficoll®-Hypaque density gradients).

b. Wash the lymphocyte population twice with sterile 1X PBS prior to use.

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

d. 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 to add 2.5 ml White Blood Cell Lysis Buffer per sample.

   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.

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

For buffy coat:

a. Prepare the buffy coat from 10–20 ml human blood by placing the sample at room temperature for 30 minutes or at 4°C overnight to allow the phases to separate.

   Alternatively, the blood may be centrifuged at 1,300 x g for 15 minutes at room temperature. The buffy coat is the interface between the plasma-containing upper phase and erythrocyte-containing lower phase.

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

c. Transfer the buffy coat to a sterile 17 x 100 mm tube capable of withstanding a centrifugal force of 12,000 x *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.

d. To the isolated buffy coat, add 5 ml sterile 1X PBS, mix gently by inversion, and centrifuge at 875 X g for 10 minutes at room temperature. Discard the supernatant.

e. 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 to add 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.

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

Quality Control

Absence of DNase contamination: Each lot of the DNA Isolation Kit for Mammalian Blood is tested to ensure the 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.

References

1. *Molecular Biology of the Cell* (1989) Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and J. Watson, p. 973–976, Garland Publishing.
2. *PCR Technology: Principles and Applications for DNA Amplification* (1989) p. 31–38, Henry A. Erlich, ed., Stockton Press.
3. Miller, S.A., Dykes, D.D. and H.F. Polesky (1988) *Nucleic Acids Research* 16(3):1215.
4. Lahiri, D.K. and Schanbel, B. (1993) *Biochemical Genetics* 31(7/8):321–329.

Troubleshooting Guide

Problem	Remedy
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 room temperature, 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 complete 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 larger number of white cells. If too many cells are present, the solution will become very viscous, and the cells will clump. • To facilitate complete lysis, samples may be incubated at 37°C for 15–30 minutes.
No protein pellet is observed following protein precipitation	• Mix the sample thoroughly by vortexing after addition of the Protein Precipitation Solution. *Recommendation*: Vortex continuously for approximately 25 seconds. • 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. • To ensure effective pelleting of protein, the samples must be spun at 12,000 x 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. Do not exceed 5 minutes of drying time under vacuum, and do not use heat when drying. To avoid these problems, air dry the samples, which helps to reduce overdrying. This method requires a longer time to complete. • Heat to 65°C to aid resuspension. Do not exceed 1 h incubation time at 65°C. Alternatively, resuspend samples overnight at 4°C.
Low DNA yields	• There was an insufficient number of leukocytes in the starting sample; increase the volume of starting sample. • Adjust the volume of lysis buffer to accommodate smaller numbers of leukocytes or smaller volumes of blood. • Incomplete white blood cell lysis: see recommendations above (under "Incomplete white blood cell lysis"). • If using blood stored for 7 days at 4°C or ≤1 month at –20°C, the expected yields will be 10–15% lower than those of freshly isolated blood.
DNA is not functional in subsequent applications (*e.g.*, the A_{260}/A_{280} ratio is too high or too low)	• $A_{260}/A_{280} < 1.6$: 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 re-precipitate 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.

*Available from Boehringer Mannheim
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