For general laboratory use.

High Pure PCR Template Preparation Kit

Version: 26

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For isolation of nucleic acids for PCR and Southern Blotting

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

Cat. No. 11 796 828 001 1 kit
up to 100 purifications

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	white	Tissue Lysis Buffer	4 M urea, 200 mM Tris, 20 mM NaCl, 200 mM EDTA, pH 7.4 (+25°C)	20 ml
2	green	Binding Buffer	6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton X-100 (v/v), pH 4.4 (+25°C)	20 ml
3	pink	Proteinase K, recombinant PCR grade	for sample lysis and inactivation of endogenous DNase	Lyophilizate
4a	black	Inhibitor Removal Buffer	5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C) final concentration after addition of ethanol.	33 ml, add 20 ml absolute ethanol
4	blue	Wash Buffer	20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) final concentration after addition of ethanol.	20 ml, add 80 ml absolute ethanol
5	colorless	Elution buffer	10 mM Tris-HCl, pH 8.5 (+25°C)	40 ml
6		High Pure Filter Tubes		Two bags with 50 polypropylene tubes with two layers of glass fiber fleece, for use of up to 700 µl sample volume
7		Collection Tubes		Eight bags with 50 polypropylene tubes (2 ml)

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

The buffers can show a slight yellow color. This will have no impact on the function of the buffer

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.

⚠ ***Please note, that improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) will adversely impact nucleic acid purification because solutions might precipitate.***

Storage Conditions (Working Solution)

Solution	Storage
Proteinase K solution	-15 to -25°C
Inhibitor Removal Buffer	+15 to +25°C
Wash Buffer	+15 to +25°C

1.3. Additional Equipment and Reagent required

Refer to the list below for additional reagents and equipment required for all isolation procedures:
- Absolute ethanol
- Absolute isopropanol
- Standard tabletop microcentrifuge capable of 13,000 *x g* centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile

For the isolation of mammalian whole blood, buffy coat, or cultured cells

- PBS*

For the isolation of mouse tail

- 1 ml disposable syringe without needle

For the isolation of nucleic acids from bacteria or yeast

- PBS*
- Lysozyme* [10 mg/ml in 10 mM Tris-HCl, pH 8.0]
- Lyticase (0.5 mg/ml)

For the isolation of nucleic acids from formalin-fixed paraffin-embedded tissue sections

- Xylene
- Ethanol, 100%, 80%, 60%, 40%

RNase treatment (optional)

- RNase, DNase-free*
- High Pure PCR Product Purification Kit*
- Heating block or water bath

1.4. Application

This kit purifies nucleic acids from different sample materials, including whole blood, cultured cells, and tissue samples. Bacteria and yeast require a specific prelysis treatment using lysozyme or lyticase. Resulting nucleic acids are ready for use in PCR and restriction digest reactions.

1.5. Preparation Time

	Whole blood and cell culture	Tissue
Total time	Approx. 20 min	Approx. 2 h
Hands-on time	Approx. 12 min	Approx. 30 min

2. How to Use this Product

2.1. Before you Begin

Sample Materials

- 200 to 300 µl mammalian whole blood
- 200 µl buffy coat
- 10^4 to 10^6 cultured mammalian cells
- 25 to 50 mg mammalian solid tissue
- 0.2 to 0.5 cm mouse tail (25 to 50 mg)
- 10^8 yeast cells
- 10^9 bacterial cells (gram positive or gram negative)
- Paraffin-embedded, fixed tissue sections

Control Reactions

⚠ ***It is the user's responsibility to implement an appropriate experiment control concept.***

General Considerations

Handling Requirements

⚠ ***Binding Buffer (bottle 2) contains guanidinium hydrochloride and Triton X-100, which are irritants. Inhibitor Removal Buffer (bottle 4a) and Wash Buffer (bottle 4) contain guanidinium hydrochloride which is an irritant.***

⚠ ***Do not let the Binding Buffer, Inhibitor Removal Buffer, or Wash Buffer touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagent, dilute the spill with water before wiping it up.***

⚠ ***Do not use any modified ethanol.***

⚠ ***Do not pool reagents from different lots.***

⚠ ***Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening store all bottles in an upright position.***

⚠ ***Do not allow the Binding Buffer, Inhibitor Removal Buffer, or Wash Buffer to mix with sodium hypchlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

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 set-up 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.
- Safety Data Sheets (SDS) are available online at dialog.roche.com, or upon request from the local Roche office.

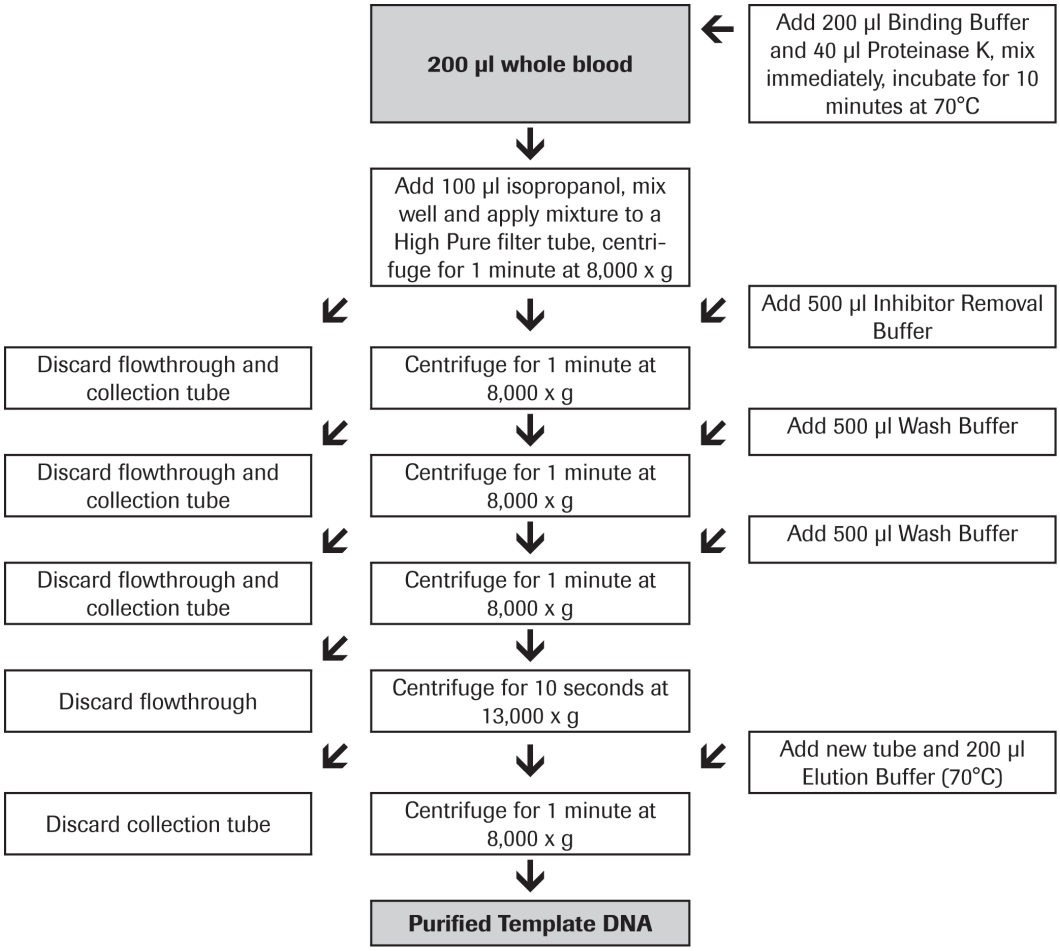
Working Solution

In addition to the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content	Reconstitution/Preparation	Storage and Stability	For use in...
Proteinase K (Vial 3; pink cap)	Dissolve Proteinase K in 4.5 ml double distilled water, aliquot solution.	▪ Store at −15 to −25°C. ▪ Stable for 12 months.	Sample Lysis and DNA Binding Protocol step 1
Inhibitor Removal Buffer (Vial 4 a; black cap)	Add 20 ml absolute ethanol to Inhibitor Removal Buffer. *Label and date bottle accordingly after adding ethanol.*	▪ Store at +15 to +25°C. ▪ Stable through the expiration date printed on kit label	Washing and Elution Protocol step 1
Wash Buffer (Vial 4; blue cap)	Add 80 ml absolute ethanol to Wash Buffer. *Label and date bottle accordingly after adding ethanol.*	▪ Store at +15 to +25°C. ▪ Stable through expiration date printed on kit label	Washing and Elution Protocol step 2 and 3

2.2. Protocols

Experimental overview



Isolation of Nucleic Acids from Mammalian Whole Blood, Buffy Coat, or Cultured Cells

Adjustment of Sample Volume

When your sample volume is less than 200 µl, increase the sample volume with PBS. Adjust the sample volume as follows:

Material	Action
Cultured cells	Centrifuge medium with cells and resuspend cell pellet in 200 µl PBS
Mammalian whole blood	When sample material is **< 200 µl**, fill up volume to 200 µl with PBS. When sample material is **> 200 µl up to 300 µl**, increase all other sample volumes accordingly.

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from:
- 200 µl mammalian blood
- 200 µl buffy coat
- 10^4 – 10^6 cultured mammalian cells

⚠ ***Before starting the purification reaction, warm up the Elution Buffer to +70°C.***

1. To a nuclease free 1.5 ml microcentrifuge tube:
   - Add 200 µl of sample material.
   - Add 200 µl lBinding Buffer.
   - Add 40 µl reconstituted Proteinase K.
   - Mix immediately and incubate at +70°C for 10 min
2. Add 100 µl isopropanol and mix well.
3. - Assemble one High Pure Filter Tube into one Collection Tube.
   - Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
   - Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   - Centrifuge 1 min at 8,000 × *g*.
4. Proceed to protocol for washing and elution.

Isolation of Nucleic Acids from Mammalian Tissue

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from 25 – 50 mg tissue.

⚠ ***Before starting the purification reaction, warm up the Elution Buffer to +70°C.***

1. To a nuclease free 1.5 ml microcentrifuge tube:
   - Add 25 – 50 mg of sample material.
   - 200 µl Tissue Lysis Buffer.
   - 40 µl reconstituted Proteinase K.
   - Mix immediately and incubate for 1 h at +55°C or until tissue is digested completely.

   ℹ *Nucleic acid yield can be increased by cutting the sample into small pieces with a scalpel before incubation.*
2. - Add 200 µl Binding Buffer.
   - Mix immediately and incubate for 10 min at +70°C.
3. - Add 100 µl isopropanol and mix well.
   - Draw a fraction of the sample into a 1 ml disposable pipette tip.

   ℹ *This procedures draws insoluble tissue particles into the pipette tip and blocks the pipette tips.*

   - Withdraw and discard the pipette, thereby removing the insoluble tissue particles
4. - Assemble one High Pure Filter Tube into one Collection Tube.
   - Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
   - Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   - Centrifuge 1 min at 8,000 × *g*.
5. Proceed to protocol for washing and elution.

Isolation of Nucleic Acids from Mouse Tail

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from 25 – 50 mg tissue.

⚠ ***Before starting the purification reaction, warm up the Elution Buffer to +70°C.***

1. To a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 0.2 – 0.5 cm (25 –50 mg) mouse tail.
   - 200 µl Tissue Lysis Buffer.
   - 40 µl reconstituted Proteinase K.
   - Mix immediately and incubate for 3 h at +55°C or until tissue is digested completely.
2. - Use 1 ml disposable syringe without needle to shear the lysed tail sample.
   - Draw the sample into the syringe and then expel it again.
   - Repeat this step twice.
3. - Add 200 µl Binding Buffer.
   - Add 100 µl isopropanol and mix well.
   - Centrifuge 5 min at 13,000 × *g*.
4. - Assemble one High Pure Filter Tube into one Collection Tube.
   - Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
   - Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   - Centrifuge 1 min at 8,000 × *g*.
5. Proceed to protocol for washing and elution.

Isolation of Nucleic Acids from Bacteria or Yeast

ⓘ *Generally, yeast cells are lysed by incubation with lyticase, however, the following yeast strains can also be lysed using lysozyme treatment:*

- Saccharomyces cerevisiae
- Aspergillus fumigatus
- Candida albicans

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from bacteria or yeast cells

⚠ **Before starting the purification reaction, warm up the Elution Buffer to +70°C.**

1. To a nuclease-free 1.5 ml microcentrifuge tube:
   - Add 200 µl bacteria or yeast cells.
   - Centrifuge for 5 min at 3,000 × *g*.
   - Resuspend cell pellet in 200 µl PBS.
2. For Bacteria:
   Add and incubate 5 µl lysozyme (10 mg/ml in 10 mM Tris-HCl, pH 8.0), and incubate 15 min at +37°C.
   For Yeast:
   Add and incubate 10 µl lyticase (0.5 mg/ml), and incubate 30 min at +37°C.
3. - Add 200 µl Binding Buffer.
   - Add 40 µl reconstituted Proteinase K
   - Mix immediately and incubate for 10 min at +70°C
   - Add 100 µl isopropanol and mix well.

4 - Assemble one High Pure Filter Tube into one Collection Tube.
- Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
- Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
- Centrifuge 1 min at 8,000 × *g*.

5 Proceed to protocol for washing and elution.

Isolation of Nucleic Acids from Formalin-Fixed Paraffin-Embedded Tissue Sections

Protocol was kindly provided by T. Fixemer, University of Homburg-Saar, Germany.

Sample Lysis and DNA Binding

For 25 - 50 mg formalin-fixed paraffin-embedded tissue section.

⚠ **Before starting the purification reaction, warm up the Elution Buffer to +70°C.**

1 Deparaffinize the tissue section in xylene for approx. 30 min.

⚠ ***Incubation time depends on the thickness of the section.***

2 Rehydrate the tissue section in a graded ethanol series for 10 s each:
- 100% ethanol (dehydration)
- 80% ethanol
- 60% ethanol
- 40% ethanol.
- Double-distilled water (rehydration) for 10 s.

⚠ ***The section should turn white after it is transferred to ethanol.***

3 - While viewing the section under a microscope, cut the desired tissue area from the rehydrated section using a scalpel.
- Transfer the sample to a clean, sterile, preweighted 1.5 ml microcentrifuge tube.
- Determine the weight of the sample.

4 To the tissue sample (25 - 50 mg):
- Add 200 µl Tissue Lysis Buffer.
- Add 40 µl reconstituted Proteinase K.
- Mix and incubate overnight at +37°C.

5 - Add 20 µl Proteinase K (reconstituted).
- Incubate for 1 - 2 h at +55°C.

⚠ ***After this incubation, no tissue particles should be visible.***

6 - Add 200 µl Binding Buffer, and mix thoroughly.
- Mix thoroughly and incubate 10 min at +70°C

7 - Add 100 µl isopropanol, and mix well.
- Use an automatic pipette to draw part of the sample into a 1 ml pipette tip.

ⓘ *This procedure draws insoluble tissue particles into the pipette tip and blocks the tip.*

- Withdraw the pipette tip and remove the insoluble tissue particles

8 - Assemble one High Pure Filter Tube into one Collection Tube.
- Pipet the remainder of the liquid sample into the upper buffer reservoir of the Filter Tube.
- Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge and centrifuge 1 min at 8,000 × *g*.

9 Proceed to protocol for washing and elution

Protocol for Washing and Elution

1 After centrifugation:
- Remove the Filter Tube from the Collection Tube. Discard the flow through liquid, and the Collection Tube.
- Assemble the Filter Tube with a new Collection Tube.
- Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 8.000 × *g*.

2 - Remove the Filter Tube from the Collection Tube. Discard the flow through liquid, and the Collection Tube.
- Assemble the Filter Tube with a new Collection Tube.
- Add 500 µl Wash Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 8,000 × *g* and discard the flow through

3 - Remove the Filter Tube from the Collection Tube. Discard the flow through liquid, and the Collection Tube.
- Assemble the Filter Tube with a new Collection Tube.
- Add 500 µl Wash Buffer to the upper reservoir of the Filter Tube.
- Centrifuge 1 min at 8,000 × *g* and discard the flow through

4 After discarding the flow through liquid:
- Centrifuge the entire High Pure assembly for 10 s at full speed.
- Discard the Collection Tube.

i This additional centrifugation step removes residual Wash Buffer.

5 To elute DNA:
- Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
- Add 200 µl prewarmed Elution Buffer to the upper reservoir of the Filter Tube.
- Centrifuge the tube assembly for 1 min at 8,000 × *g.*

6 The microcentrifuge tube now contains the eluted DNA.
Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or -15 to -25°C for later analysis.
To add an RNase digestion, go to the next section, “RNase Treatment”.

Related Procedures

RNase Treatment

To remove RNA from the DNA template before PCR, treat samples as follows:

1 Add RNase to the eluted nucleic acids, and incubate as indicated below.

2 After treatment, the RNase can be removed from the DNA with the High Pure PCR Product Purification Kit*.

Amount of cultured mammalian cells	Amount of RNase	Incubation time/temperature
10^6	0.5 µl	15 min at +15 to +25°C or +37°C

LightCycler® PCR Sample Preparation

The High Pure PCR Template Preparation Kit has been evaluated using LightCycler®PCR for whole human blood and cultured K-562 cells. Amplification was performed in LightCycler® capillaries using the SYBR Green I and HybProbe Probe detection format.
The following tables indicates the range of sample volume and concentration, as well as expected results.

Sample	Volume/Amount Range	Typical conc. /range [ng/µl]	Eluate use in LightCycler® PCR [µl]
Human whole blood	1 - 50 µl[1)]	0.5 - 25	1 - 5 [0.5 ng - 125 ng]
Cultured cells, K-562	100 - 10^5 cells	0.01 - 10	1 - 5 [0.01 ng- 50 ng]

(1) **Yields may vary between blood donors due to varying numbers of leukocytes.**

Procedure Modification

Whole blood and cultured cells have been used for sample preparation for LightCycler® PCR. However a different elution volume of 50 µl was used to increase nucleic acid concentration when testing very low level sample amounts. For larger sample amounts, the standard elution volume of 200 µl is appropriate.

Parameter	Sample Material	SYBR Green I Min	Max	HybProbes Min	Max
Cyclophilin A	blood	0.005 µl	100 µl	0.005 µl	100 µl
ß-globin	cells	1[b)]	10^4	1[b)]	10^5

The above table shows the lowest and highest sample amounts tested using the LightCycler® System with whole blood and cultured cells as sample material. The parameters human cyclophilin A and ß-globin were investigated. The table shows blood volumes and cell numbers used in a 20 µl reaction, when 5 µl (Min) and 50 µl (Max) elution volume was used during High Pure purification.

3. Results

Purity

Purified nucleic acids are free of other cellular components and DNA polymerase inhibitors.

RNA can be removed from purified NA with an optional RNase digestion.

Expected Yield

Variable depending on sample type. The table below shows experimental results:

Sample	Amount	Yield [µg] Total Nucleic Acids
Human Whole Blood	200 µl[(1)]	3 - 6
Buffy Coat	200 µl[(1)]	20
Cultured cells, K-652	10^6 cells[(2)]	15 - 20
Calf thymus	25 mg	5 - 10
Mouse tail	0.2 - 0.5 cm (25 - 50 mg)	5 - 10
Bacterial cells	10^9	1 - 3
Yeast	10^8	10 - 13

(1) Typical volume is 200 µl and the volume is 300 µl. Yields may vary between different blood donors due to varying numbers of leukocytes.

(2) Maximum cell number is 1×10^6, however this high cell number may produce a reduced yield.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity.	Kit stored under suboptimal conditions.	Store kit at +15 to +25°C after arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
		Store reconstituted reagents at either +2 to +8°C or -15 to -25°C (as indicated in the Instruction for Use).
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer.	Before use, add absolute ethanol to the buffers.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Label the Wash Buffer vial and Inhibitory Removal Buffer vial appropriately, to indicate whether ethanol has been added.
	Reagents and samples not completely mixed.	Mix the sample tube completely, after addition of each reagent.
Low recovery of nucleic acids after elution.	Suboptimal reagent has been used for elution. Alkaline pH is required for optimal elution.	Do not use water to elute nucleic acids from Filter Tube.
		Use the Elution Buffer in the kit.
	Smaller amounts of sample material used than specified.	Use Poly(A) as carrier RNA.
		Dissolve 4 mg Poly(A) carrier RNA in 1 ml sterile double-distilled water.
		Prepare 50 µl aliquots which will be sufficient for 10 samples. Store the aliquots at -15 to -25°C.
Incomplete or no restriction enzyme cleavage of product.	Glass fibers which can co-elute with the nucleic acid may inhibit enzyme reactions.	After elution step is finished, remove High Pure Filter from tube containing eluted sample and spin this sample tube for 1 minute at maximum speed.
		Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Absorbency (A_{260}) reading of product too high.	Glass fibers which can co-elute with nucleic acid, scatter light.	See suggestions under “Incomplete or no restriction enzyme cleavage of product” above.
Purified DNA sample cannot easily be loaded into the well of an agarose gel, but instead “pops out” of the well as it is loaded.	Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.	After the last wash step, make certain flow through solution containing Wash Buffer does not contact the bottom of the High Pure Filter Tube.
		If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and re-centrifuge for 30 seconds.

Observation	Possible cause	Recommendation
Low yield.	Proteinase K not completely solubilized.	To solubilize the lyophilized Proteinase K completely: ① Pipet 4.5 ml of double-distilled water into the glass vial containing lyophilized Proteinase K. ② Add the rubber stopper and invert the vial until all the lyophilizate, including lyophilizate on the rubber stopper, is dissolved. ③ Aliquot the reconstituted enzyme, labeling each aliquot with the date of reconstitution, and store at -15 to -25°C. Reconstituted Proteinase K is stable for 12 months at -15 to -25°C.
	Incomplete lysis.	Add reconstituted Proteinase K to the sample and mix immediately.
		Mix lysate completely with isopropanol before adding this mixture to the High Pure Filter Tube.
Low yield from tissue.	Incomplete Proteinase K digestion.	Cut tissue into small pieces before digestion and lysis.
		Increase incubation time with Proteinase K in either of two ways: ① Incubate tissue with Proteinase K over-night. ② Incubate with Proteinase K for 3 - 4 h, then add a fresh aliquot of Proteinase K (30 µl) and incubate another 1 - 2 h. ⚠ ***To accommodate increased volume of sample and enzyme, use 230 µl Binding Buffer. Instead of the 200 µl indicated in Step 2 of the procedure described under 'Isolation of Nucleic Acids from Mammalian Tissues'***
Low yield from bacteria or yeast.	Bacterial and yeast cells are not lysed efficiently with lysozyme or lyticase, respectively.	Make sure cells are lysed by lysozyme or lyticase.
		The Instruction for Use lists strains (known to be lysed by these enzymes).
		Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling, or repeated freeze-thawing.
Degraded DNA from tissue samples.	Nuclease activity in un-lysed tissue.	Tissue should be frozen (-15 to -25°C) from the time of harvest until lysis.
		Use smaller pieces of tissue (20 - 40 mg) or homogenize tissue sample.
Eluate from blood is still slightly colored.	Incomplete wash.	① Wash Filter Tube until flow through is colorless. ② Repeat purification using 200 µl eluate from the first purification as starting material; add 200 µl Binding Buffer, mix well, then add 100 µl isopropanol. ③ Follow the same procedure, starting with the application of the sample to the High Pure Filter Tube (Step 4). ⚠ ***Omit Proteinase K digestion and +70°C incubation.***

5. Additional Information on this Product

5.1. Test Principle

Cells are lysed using a short incubation with Proteinase K in the presence of a chaotropic salt (guanidine-HCl). Cellular nucleic acids (NA) bind selectively to special glass fibers pre-packed in the High Pure Purification Filter Tube. Bound NA is purified in a series of rapid “wash-and-spin” steps to remove contaminating cellular components. A specially formulated Inhibitor Removal Buffer has been included for use with sample material treated with 100 U/ml of heparin. Low salt elution is used to release NA from the glass fiber. This simple method eliminates the need for organic solvent extractions and DNA precipitation, ideal for rapidly purifying many samples simultaneously.

① Blood, cells or tissue are lysed by incubation with a special Lysis Buffer and Proteinase K.

② Nucleic acids are bound to the glass fibers pre-packed in the High Pure Filter Tube.

③ Bound nucleic acids are washed with a special Inhibitor Removal Buffer to get rid of PCR inhibitory contaminants.

④ Washing of bound nucleic acids, purification from salts, proteins and other cellular impurities.

⑤ Purified nucleic acids are recovered using the Elution Buffer.

5.2. Quality Control

DNA is isolated from 25 mg of calf thymus, 1 × 10^6 K-562 cells and 200 µl of EDTA whole blood. Yield is measured using spectrophotometry (OD) for DNA from tissue and cell samples. Nucleic acid quality is assessed using an Expand Long Range PCR with a 9.3 kb amplification product. Real-time PCR is performed on human blood research samples using kits for Factor V Leiden and CycA.

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

Removal of redundant centrifugation step in chapter 2.2. on page 9.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Poly(A)	100 mg	10 108 626 001
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
RNase, DNase-free	500 µg, 1 ml	11 119 915 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
Lysozyme	10 g	10 837 059 001

6.4. Trademarks

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6.5. License Disclaimer

For patent license limitations for individual products please refer to:
http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet (SDS)

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

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.
To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

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