For general laboratory use.
FOR *IN VITRO* USE ONLY.

High Pure PCR Template Preparation Kit

Version July 2006

For Isolation of Nucleic Acids for PCR and Southern Blotting

Cat. No. 11 796 828 001 Kit for 100 isolations

Store the kit at +15 to +25°C.

⚠ If properly stored, all kit components are stable through the expiration date printed on the label.

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PROTOCOLS

1. What this Product Does

Number of Tests The kit is designed for 100 isolation reactions.

Kit Contents

⚠ 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 waterbath until the precipitates have dissolved.

Vial/Cap	Label	Contents / Function
1 white	Tissue Lysis Buffer	• 20 ml • [4 M urea, 200 mM Tris, 20 mM NaCl, 200 mM EDTA, pH 7.4 (25°C)]
2 green	Binding Buffer	• 20 ml • [6 M guaninidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% Triton X-100 (v/v), pH 4.4 (25°C)]
3 pink	Proteinase K, recombinant PCR grade	• Lyophilizate • For sample lysis and inactivation of endogenous DNase
4a black	Inhibitor Removal Buffer	• 33 ml, add 20 ml absolute ethanol • [5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (25°C) final concentration after addition of ethanol]
4 blue	Wash Buffer	• 20 ml, add 80 ml absolute ethanol • [20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (25°C) final concentrations after addition of ethanol]
5 colorless	Elution Buffer	• 40 ml • [10 mM Tris-HCl, pH 8.5 (25°C)]
	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.
	Collection Tubes	Eight bags with 50 polypropylene tubes (2 ml).

Storage and Stability

The High Pure PCR Template Preparation Kit components must be stored at +15 to +25°C. Kit components are guaranteed to be stable through 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.
Therefore, High Pure isolation kits are always shipped at +15 to +25°C.

After dissolution of Proteinase K the solution should be aliquoted and stored at −15 to −25°C. The solution is stable at −15 to −25°C for up to 1 year.

Additional Equipment and Reagents 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 × *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

** available from Roche Applied Science*

Application

This kit is designed to purify nucleic acids from different sample materials, including whole blood, cultured cells, and tissue samples. Bacteria and yeast require a specific prelysis treatment with lysozyme or lyticase. The resulting nucleic acids are ready for use in PCR and restriction-digestion reactions.

Assay 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

Precautions

I) Handling Requirements

- Complete each phase of the PCR workflow before proceeding to the next phase. For example, you should finish PCR sample preparation before starting PCR set-up. Sample prep, PCR set-up and the PCR run itself should also be performed in separate locations.
- Do not pool reagents from different lots.
- Do not use a kit after its expiration date has passed.
- 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.

II) 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 has to optimize pathogen inactivation 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 coates and eye protection when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or ribonuclease. 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 test reagents.

III) Waste Handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Material Safety Data Sheets (MSDS) are available on the Roche Applied Science home page or upon request from the local Roche office.

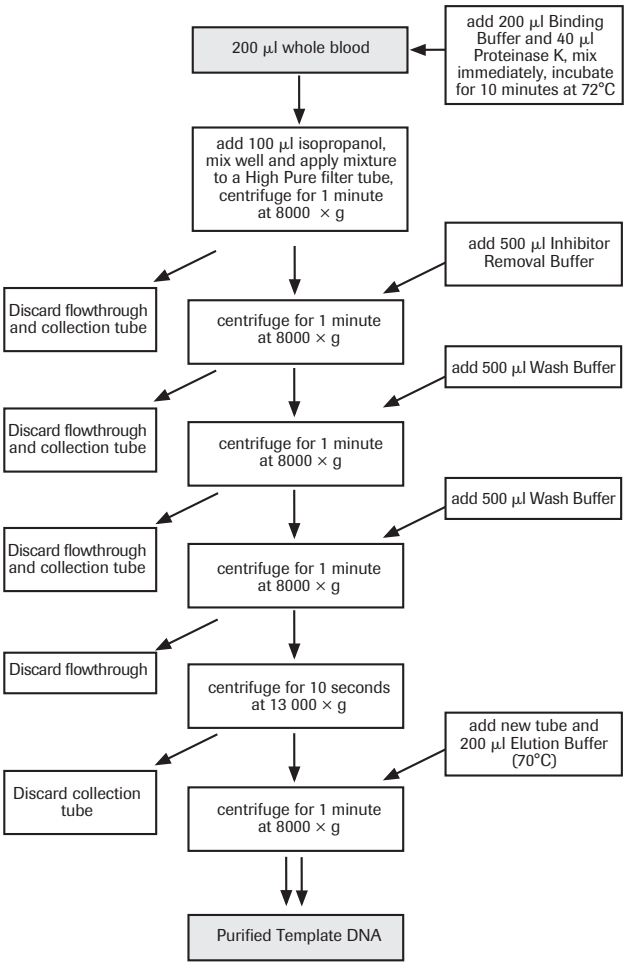
Sample Material

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

Preparation of Working Solutions

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

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


200 µl whole blood
add 200 µl Binding Buffer and 40 µl Proteinase K, mix immediately, incubate for 10 minutes at 72°C
add 100 µl isopropanol, mix well and apply mixture to a High Pure filter tube, centrifuge for 1 minute at 8000 × g
add 500 µl Inhibitor Removal Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8000 × g
add 500 µl Wash Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8000 × g
add 500 µl Wash Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8000 × g
Discard flowthrough
centrifuge for 10 seconds at 13 000 × g
add new tube and 200 µl Elution Buffer (70°C)
Discard collection tube
centrifuge for 1 minute at 8000 × g
Purified Template DNA

Adjustment of sample volume

If your sample volume is less than 200 µl, the sample volume should be expanded with PBS*. Adjust the sample volume as follows:

Material	Action
cell culture cells	centrifuge medium with cells and resuspend cell pellet in 200 µl PBS.
mammalian whole blood	If sample material is < **200 µl** fill up volume to 200 µl with PBS If sample material is **> 200 µl up to 300 µl** increase all volumes accordingly

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from:

- 200 µl mammalian blood,
- 200 µl buffy coat,
- or 10^4 - 10^6 cultured mammalian cells

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

❶ To a nuclease free 1.5 ml microcentrifuge tube
- Add 200 µl of sample material.
- Add 200 µl Binding Buffer.
- Add 40 µl Proteinase K (reconstituted)
- Mix immediately and incubate at 70°C for 10 min.

❷ Add 100 µl Isopropanol and mix well.

❸
- Insert one High Filter Tube in one Collection Tube.
- Pipet the sample into the upper buffer reservoir of the Filter Tube.
- Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge.
- Centrifuge 1 min at 8,000 × *g*.

❹ Proceed to Washing and Elution section 2.8.

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.

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

🔍 The yield of nucleic acids can be increased by cutting the sample with a scalpel in small pieces before incubation.

❷
- Add 200 µl Binding Buffer.
- Mix immediately and incubate for 10 min at 70°C.

❸
- Add 100 µl isopropanol and mix well.
- Draw part of the sample into a 1 ml disposable pipette tip.

🔍 This treatment draws insoluble tissue segments into the pipette tip and blocks it.
- Withdraw and discard the pipette tip, carrying the insoluble tissue segments with it.

❹
- Insert one High Filter Tube in 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 table-top centrifuge.
- Centrifuge 1 min at 8,000 × *g*.

❺ Proceed to Washing and Elution section 2.8.

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from 0.2 - 0.5 cm (25 - 35 mg)

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

❶	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 Proteinase K (reconstituted). • Mix immediately and incubate for 3 h at 55°C or until tissue is digested completely.
❷	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 the step twice.
❸	• Add 200 µl Binding Buffer. • Add 100 µl isopropanol and mix well. • Centrifuge 5 min at 13,000 × *g*.
❹	• Insert one High Filter Tube in one Collection Tube. • Pipet the liquid sample into the upper buffer reservoir of the Filter Tube. • Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge. • Centrifuge 1 min at 8,000 × *g*.
❺	Proceed to Washing and Elution section 2.8.

Generally, yeast cells are lysed by incubation with Lyticase, but some yeast strains can also be lysed by Lysozyme treatment:

- *Saccharomyces cerevisiae*
- *Aspergillus fumigatus*
- *Candida albicans*

Sample Lysis and DNA Binding

Protocol for the isolation of nucleic acids from 10^9 bacteria or 10^8 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. To the sample material
   - Add 200 µl Binding Buffer.
   - Add 40 µl Proteinase K (reconstituted).
   - Mix immediately and incubate for 10 min at 70°C.
4. Add 100 µl Isopropanol and mix well.
5. - Insert one High Filter Tube in one Collection Tube.
   - Pipet the liquid sample into the upper buffer reservoir of the Filter Tube.
   - Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge.
   - Centrifuge 1 min at 8,000 × *g*.
6. Proceed to Washing and Elution section 2.8.

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.

❶ Soak the tissue section in xylene to deparaffinize for approx. 30 min.

⚠ Incubation time depends on the thickness of the section.

❷ Incubate the tissue section in a graded ethanol series for 10 s each:

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

- 100% ethanol (dehydration)/ 80% ethanol/ 60% ethanol/ 40% ethanol
- Double distilled water (rehydration) for 10 s.

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

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

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

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

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

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

⊘ This treatment draws insoluble tissue segments into the pipette tip and blocks it.

- Withdraw the pipette tip, carrying the insoluble tissue segments with it.

❽
- Insert one High Filter Tube in 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*.

❾ Proceed to Washing and Elution section 2.8.

❶ After centrifugation:
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
- Combine 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*.

❷
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
- Combine 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 flowthrough.

❸
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube.
- Combine 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 flowthrough.

❹ After discarding the flowthrough liquid:
- Centrifuge the entire High Pure assambly for additional 10 s at full speed.
- Discard the Collection Tube.

The extra centrifugation time ensures removal of residual Wash Buffer.

❺ To elute the 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*.

❻ The microcentrifuge tube now contains the eluted plasmid 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.
Or if you want to add an RNase digestion see related procedures 2.9.

RNase treatment

If you wish to remove RNA from the template before PCR treat your sample as follows:

❶	Add to the eluted nucleic acids RNase and incubate as appropriate (see table below).
❷	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

Sample Material

The High Pure PCR Template Preparation Kit has been evaluated for sample preparation for LightCycler® PCR with whole human blood and cultered K562 cells as sample material. Amplification has been performed in LightCycler® capillaries using SYBR Green I and HybProbe detection format. The following tables give information about the range of sample volume applied, modifications in the sample preparation procedure and expected results.

Sample	Volume/Amount Range	Typical conc./ range [ng/µl]	Eluate use in LightCycler® PCR (µl)
Human whole blood	1 – 50 µl[a)]	0.5 – 25	1 – 5 [0.5 ng – 125 ng]
Cultured cells, K562	100 – 10^5 cells	0.01 – 10	1 – 5 [0.01 ng– 50 ng]

[a)] Yields may vary between different blood donors because they may have different amounts of leukocytes.

Procedure Modification

The standard procedure for whole blood and cultured cells has been used for sample preparation for LightCycler® PCR except the elution volume was set to 50 µl in order to increase nucleic acid concentration for minute sample amounts. For larger sample amounts the standard elution volume of 200 µl can be applied.

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

[b)] The used quantities of eluate correspond to these calculated amounts.

The above table shows the minimal and maximal sample amounts which have been used for LightCycler® PCR with whole blood and cultured cells as sample material. The parameters human cyclophilin A and ß-globin have been investigated. All values are for a 20 µl LightCycler® PCR when 5 µl of 50 µl total eluate is applied.

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, K562	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, maximum volume is 300 µl. Yields may vary between different blood donors because they may have different amounts of leukocytes.

[2)] The maximum possible cell number is 1×10^6 but may already lead to a reduced yield, *i.e.* you cannot assume a linear 10fold increase in DNA yield when increasing the cell number from 10^5 to 10^6 cells.

	Possible Cause	Recommendation
Low nucleic acid yield or purity.	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times upon 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. • After any lyophilized reagent is constituted aliquot it and store the aliquot at either +2 to +8°C or −15 to −25°C (as directed in the instruction manual).
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer	1. Add absolute ethanol to the buffers before using. 2. After adding ethanol, mix the buffers well and store at +15 to +25°C. 3. Always mark Wash Buffer vial and Inhibitory Removal Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Low recovery of nucleic acids after elution.	Non-optimal 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.	Please use Poly (A) as carrier RNA. • Dissolve 4 mg Poly(A) carrier RNA in 1 ml sterile double dist. H_2O . • 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 coelute with the nucleic acid may inhibit enzyme reactions.	1. After elution step is complete, remove High Pure Filter from tube containing eluted sample and spin this sample tube for 1 minute at maximum speed. 2. 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 coelute with nucleic acid, scatter light.	See suggestions under "Incomplete or no restriction enzyme cleavage of product" above.

	Possible Cause	Recommendation
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.	1. After the last wash step, make certain flowthrough solution containing Wash Buffer does not contact the bottom of the High Pure Filter Tube. 2. If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and re-centrifuge for 30 seconds.
Low yield.	Proteinase K not completely solubilized.	To solubilize the lyophilized Proteinase K completely: 1. Pipette 4.5 ml of double distilled water into the glass vial containing lyophilized Proteinase K. 2. Stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. 3. Aliquot the reconstituted enzyme, mark each aliquot with the date of reconstitution, and store at -15 to -25°C. Reconstituted Proteinase K is stable for 12 months when stored properly.
	Incomplete lysis.	• After adding Proteinase K to the sample mix immediately. • Always mix lysate well with isopropanol before adding 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 overnight. 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 (sample and enzyme), use 230 µl Binding Buffer instead of 200 µl in step 2 of procedure 2.4.

	Possible Cause	Recommendation
Low yield from bacteria or yeast.	Cells are not lysed efficiently with lysozyme (bacteria) or lyticase (yeast).	• Make sure that your cells can be lysed by lysozyme or lyticase. • The instruction manual lists some recommended strains (which are known to be lysed by the enzymes). • Use alternative lysis procedures, such as mechanical grinding, vortexing with glass beads, boiling or repeated freeze-thaw.
Degraded DNA from tissue samples.	Nuclease activity in unlysed tissue.	• Tissue should be frozen (−15 to −25°C) from the time of harvest until the lysis procedure starts. • Use only small pieces of tissue (20 - 40 mg), in the procedure or homogenize tissue sample.
Eluate from blood is still slightly colored.	Incomplete wash.	1. Wash Filter Tube until flowthrough is colorless. 2. Repeat purification by using 200 µl eluate from the first purification round as starting material; add 200 µl Binding Buffer, mix well, then add 100 µl isopropanol. 3. Follow corresponding 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

How this Product Works

Cells are lysed during a short incubation with Proteinase K in the presence of a chaotropic salt (guanidine-HCl), which immediately inactivates all nucleases. 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 special Inhibitor Removal Buffer has been included which allows even the application of heparinized sample material with - 100 U/ml of Heparin. Finally, low salt elution releases the NA from the glass fiber. This simple method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

Test Principle

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.

References

1 Vogelstein, B. et al. (1979) Preparative and analytical purification of DNA from agarose *Proc. Natl. Acad. Sci. USA* **76**, 615-619.

2 Bandea, CI. et al. (2001) Typing of Chlamydia trachomatis strains from urine samples by amplification and sequencing the major outer membrane protein gene (omp1) *Sex Transm Infect.* **77**(6),419-22.

3 Lee, T.L. et al. (2001) Inverse association between cyclooxygenase-2 overexpression and microsatellite instability in gastric cancer. *Cancer Letters* **168**, 133-140.

4 Reischl, U. et al. (2003) Comparison of two DNA targets for the diagnosis of Toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. *BMC Infectious Diseases* **3**,7.

5 Mayall, F. et al. (2003) The detection of Simian virus 40 in mesotheliomas from New Zealand and England using real time FRET probe PCR protocols *Journal of Clinical Pathology* **56**, 728-730.

6 Kámory, E. et al. (2004) Rapid Detection of Cystic Fibrosis Transmembrane Conductance Regulator Gene IVS8 5T Variant by Real-Time PCR *Clinical Chemistry* **50**, 1837-1839.

7 Slowik, A. et al. (2004) II Genotype of the Angiotensin-Converting Enzyme Gene Increases the Risk for Subarachnoid Hemorrhage From Ruptured Aneurysm *Stroke* **35**,1594-1597.

Quality Control

At least 5 µg of DNA is isolated from 25 mg calf thymus when treated as described in the protocol for tissue samples. After RNase digestion the DNA concentration is determined. 100 ng of the nucleic acid is used with specific primer for amplification of a 1.5 kb DNA fragment of 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 15 kb amplification product.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered stages labeled ① , ②, etc	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, etc	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Versions

Proteinase K is now available as recombinant enzyme. For proper storage and usage, please see chapter 1 of this manual.

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com and our Special Interest Sites including:

- Nucleic Acid Isolation and Purification: http://www.roche-applied-science.com/napure
- PCR - Innovative Tools for Amplification: http://www.roche-applied-science.com/pcr

Associated Kits

Product	Pack Size	Cat. No.
Expand Long Template PCR System	100 U 500 U 10 × 250 U	11 681 834 001 11 681 842 001 11 759 060 001
Expand 20 kb^{PLUS} PCR System	100 U	11 811 002 001
PCR Core Kit	1 kit (100 PCR reactions)	11 578 553 001
PCR Core Kit^{PLUS}	1 kit (100 PCR and UNG reactions)	11 585 541 001
LightCycler® DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	12 015 102 001 12 158 825 001
LightCycler® FastStart DNA Master HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 003 248 001 12 239 272 001
LightCycler® FastStart DNA Master^{PLUS} HybProbe	1 kit (96 reactions) 1 kit (480 reactions)	03 515 575 001 03 515 567 001
LightCycler® DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	12 015 099 001 12 158 817 001
LightCycler® FastStart DNA Master SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 003 230 001 12 239 264 001
LightCycler® FastStart DNA Master^{PLUS} SYBR Green I	1 kit (96 reactions) 1 kit (480 reactions)	03 515 869 001 03 515 885 001
LightCycler® Control Kit DNA	1 kit (50 reactions)	12 158 833 001
High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001

	Product	Pack Size	Cat. No.
Single reagents	Lysozyme	10 g	10 837 059 001
	Poly (A)	100 mg	10 108 626 001
	Buffers in a Box Premixed PBS buffer(10×)	4 l	11 666 789 001
	RNase, DNase-free	500 g (1 ml)	11 119 915 001
	Taq DNA Polymerase 5 U/µl 1 U/µl	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U 250 U 4 × 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001 11 647 679 001 11 647 687 001
	Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001

6.4 Trademarks

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