For general laboratory use.

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

High Pure PCR Template Preparation Kit

Version 18

Content version: January 2012

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.

⚠ When properly stored, kit components are stable until the expiration date printed on the label.

www.roche-applied-science.com

Table of Contents

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 water bath 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 until the expiration date printed on the label.

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

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 Guidelines

- Complete each step of the PCR workflow before proceeding to the next step. For example, prepare PCR samples before starting PCR setup. Sample preparation, PCR setup, and PCR should 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) contains guanidinium hydrochloride which is an irritant. Do not let the Binding Buffer or Inhibitor Removal 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 titer of potential pathogens in sample material can vary, perform pathogen inactivation and 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.
- 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 Homepage or upon request from your local Roche representative.

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

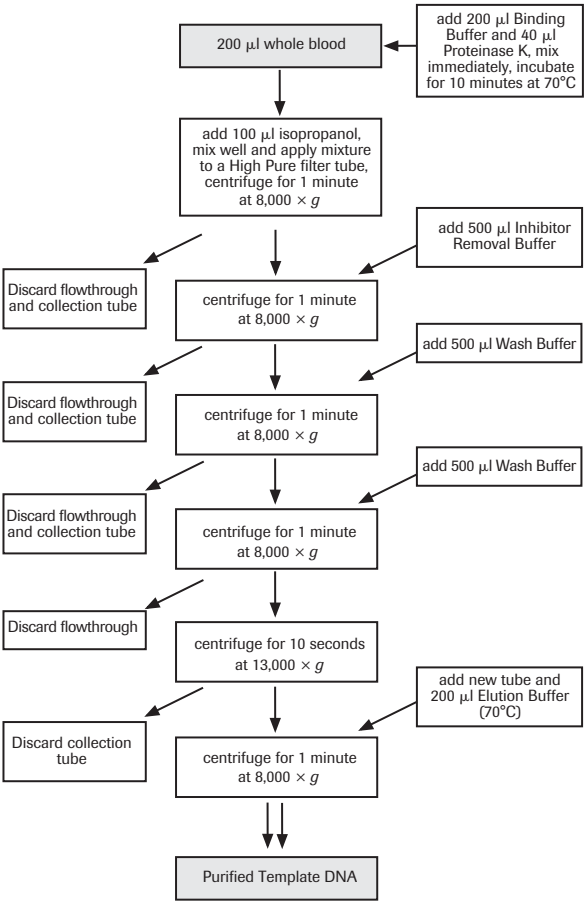
Preparation of Working Solutions

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

Controls

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


200 µl whole blood
add 200 µl Binding Buffer and 40 µl Proteinase K, mix immediately, incubate for 10 minutes at 70°C
add 100 µl isopropanol, mix well and apply mixture to a High Pure filter tube, centrifuge for 1 minute at 8,000 × g
add 500 µl Inhibitor Removal Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8,000 × g
add 500 µl Wash Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8,000 × g
add 500 µl Wash Buffer
Discard flowthrough and collection tube
centrifuge for 1 minute at 8,000 × 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 8,000 × g
Purified Template DNA

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
cell culture 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.

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

❷ Add 100 µl isopropanol and mix well.

❸
- Assemble one High Filter Tube into 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, as described 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 reconstituted Proteinase K.
- Mix immediately and incubate for 1 h at 55°C or until tissue is digested completely.

Nucleic acid yield can be increased by cutting the sample into small pieces with a scalpel 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 a fraction of the sample into a 1 ml disposable pipette tip.

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.

❹
- Assemble one High 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 table-top centrifuge.
- Centrifuge 1 min at 8,000 × *g*.

❺ Proceed to washing and elution, in 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 reconstituted Proteinase K. ▪ 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 this step twice.
❸	▪ Add 200 µl Binding Buffer. ▪ Add 100 µl isopropanol and mix well. ▪ Centrifuge 5 min at 13,000 × *g*.
❹	▪ Assemble one High Filter Tube into 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, in section 2.8.

Generally, yeast cells are lysed by incubation with lyticase, but these 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 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 reconstituted Proteinase K.
   - Mix immediately and incubate for 10 min at +70°C.
4. Add 100 µl isopropanol and mix well.
5. - Assemble one High Filter Tube into 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, in 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 Deparaffinize the tissue section in xylene for approx. 30 min.

⚠ Incubation time depends on the thickness of the section.

❷ 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.

❸
- 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.

❹ To the tissue sample (25 – 50 mg):
- Add 200 µl Tissue Lysis Buffer.
- Add 40 µl reconstituted Proteinase K.
- 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, no 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 procedure draws insoluble tissue particles into the pipette tip and blocks the tip.
- Withdraw the pipette tip and remove the insoluble tissue particles.

❽
- Assemble one High 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 table-top centrifuge and centrifuge 1 min at 8,000 × *g*.

❾ Proceed to washing and elution, in section 2.8.

❶ After centrifugation:
- Remove the Filter Tube from the Collection Tube. Dscard 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*.

❷
- 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.

❸
- 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.

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

  This additional centrifugation steps removes residual Wash Buffer.

❺ 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*.

❻ 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"..

RNase treatment

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

❶	Add RNase to the eluted nucleic acids, and incubate as indicated 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 using LightCycler® PCR for whole human blood and cultured K562 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[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 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 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	SYBR Green I Max	HybProbes Min	HybProbes 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 for 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 comtaminants and DNA polymerase inhibitors.

RNA can be removed from purified DNA using 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 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 but this high cell number may produce a reduced yield.

	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 manual).
	Ethanol not added to Wash Buffer and Inhibitory Removal Buffer	1. Before use, add absolute ethanol to the buffers. 2. After adding ethanol, mix the buffers well and store at +15 to +25°C. 3. 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.	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 flow through 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 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 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 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 of sample and enzyme, use 230 µl Binding Buffer instead of the 200 µl indicated in step No. 2 of the procedure described in section 2.4.

	Possible Cause	Recommendation
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 manual 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 unlysed 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 No. 4). ⚠ Omit Proteinase K digestion and +70°C incubation.

5. Additional Information on this Product

How this Product Works

Cells are lysed using 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 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.

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

DNA is isolated from 25 mg of calf thymus, 1 × 10^6 K562 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

Text Conventions To make information consistent and easy to read, 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

- Editorial changes

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 Homepage www.roche-applied-science.com and the following Special Interest Sites:

- 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 Disclaimer of License

NOTICE TO PURCHASER: This is a product licensed under patents owned by Qiagen.

6.5 Trademarks

EXPAND, FASTSTART, HIGH PURE, HYBPROBE, and LIGHTCYCLER, are trademarks of Roche.

Tween is a registered trademark of ICI Americans, Inc.

Ltd Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology

All other product names and trademarks are the property of their respective owners.

Regulatory Disclaimer: For general laboratory use.

Contact and Support

If you have questions or experience problems with this or any Roche Applied Science (RAS) product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the world-wide research community.

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

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country.
Country-specific contact information will be displayed.
On the Roche Applied Science home page select **Printed Materials** to find:

- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.

0112.**11786318001**®

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany