


# High Pure PCR Template Preparation Kit

for preparation of up to 100 nucleic acid samples

Cat. No. 11 796 828 001

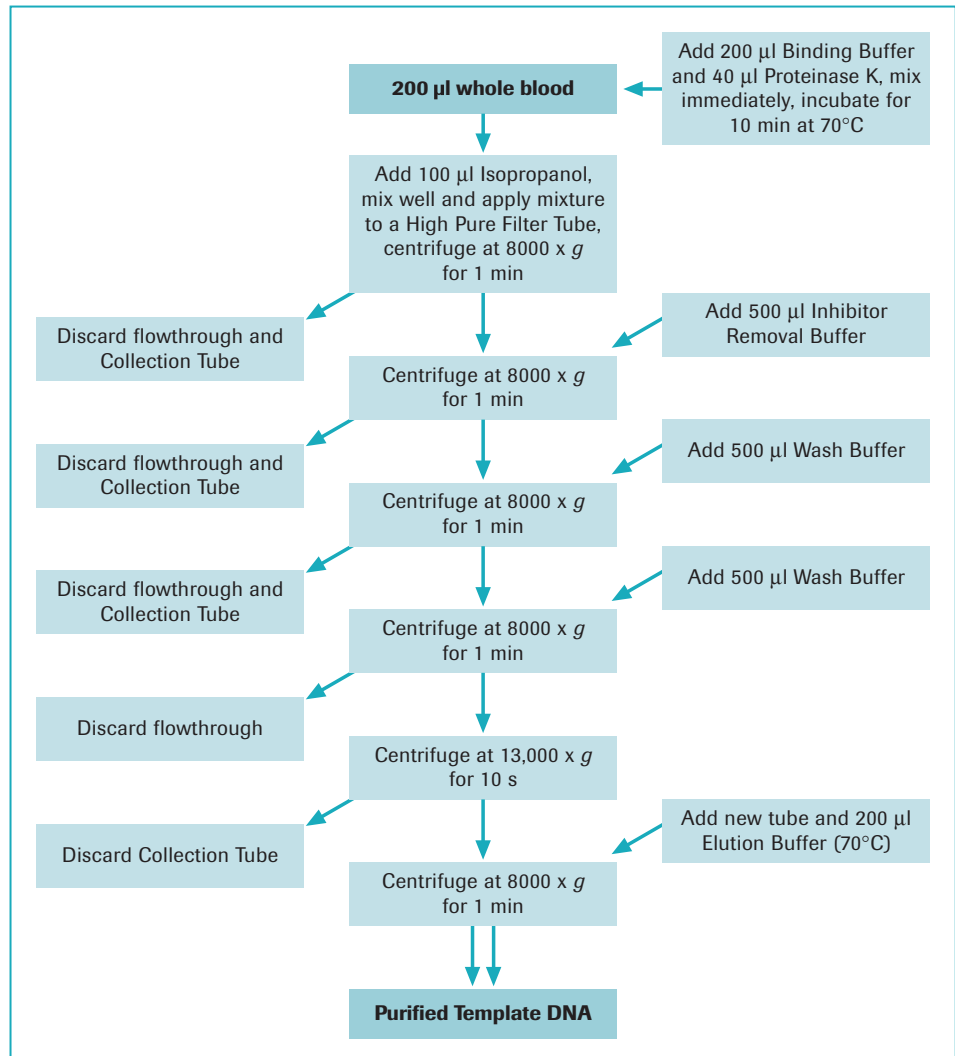
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<b>Principle</b>	Cells are lysed during a short incubation with Proteinase K in the presence of a chaotropic salt (guanidine HCl), which immediately inactivates all nucleases. Cellular nucleic acids bind selectively to glass fiber fleece in a special centrifuge tube. The nucleic acids remains bound while a series of rapid “wash-and-spin” steps remove contaminating small molecules. Finally, low salt elution removes the nucleic acids from the glass fiber fleece. The process does not require precipitation, organic solvent extractions, or extensive handling of the nucleic acids.
<b>Starting material</b>	<ul style="list-style-type: none"> <li>● 200 – 300 µl human whole blood (research samples), containing any anticoagulant</li> <li>● 200 µl buffy coat (research samples)</li> <li>● 10<sup>4</sup> – 10<sup>8</sup> cultured mammalian cells</li> <li>● 25 – 50 mg mammalian tissue</li> <li>● 0.2 – 0.5 cm mouse tail (25 – 50 mg)</li> <li>● 10<sup>8</sup> yeast cells</li> <li>● 10<sup>9</sup> bacteria cells (gram positive or gram negative)</li> <li>● Paraffin-embedded, fixed tissue sections</li> </ul>
<b>Application</b>	<ul style="list-style-type: none"> <li>● Preparation of high molecular weight nucleic acids (30 – 50 kb), which may be used directly in standard PCR, long template PCR, or Southern blots</li> </ul>
<b>Time required</b>	<ul style="list-style-type: none"> <li>● Total time: approx. 20 min (for whole blood or cultured cells)</li> <li>● Hands-on time: approx. 12 min (for whole blood or cultured cells)</li> </ul>
<b>Results</b>	<ul style="list-style-type: none"> <li>● Yield: Variable, depending on sample type (See the table under Part IV of “How to use the kit” in this article)</li> <li>● Purity: Purified nucleic acid is free of other cellular components and DNA polymerase inhibitors</li> </ul> <p> <i>RNA can be removed from the purified nucleic acids with an optional RNase digestion (see page 24).</i></p>
<b>Benefits</b>	<p><b>Saves time</b>, because the kit can produce multiple PCR templates in minutes</p> <p><b>Improves PCR reproducibility and reliability</b>, because the kit removes inhibitors that might cause PCR templates to behave unpredictably</p> <p><b>Minimizes DNA loss</b>, because the kit completely removes contaminants without precipitation or other handling steps that can lead to lost or degraded DNA</p> <p><b>Ideal for a wide variety of research projects</b>, because one kit can purify nucleic acids from many sources</p>

## How to use the kit

### I. Flow diagram

The chart shows as an example protocol steps for the sample material whole blood.



### II. Kit contents

- Tissue Lysis Buffer with 4 M urea (20 ml)
- Binding Buffer with 6 M guanidine HCl (20 ml)
- Proteinase K, lyophilized recombinant
  - ! Dissolve in 4.5 ml double-distilled H<sub>2</sub>O before use. Store aliquoted at -15 to -25°C.
- Inhibitor Removal Puffer (33 ml)
  - ! Add 20 ml absolute ethanol before use.
- Wash Buffer (20 ml)
  - ! Add 80 ml absolute ethanol to Wash Buffer before use.

- Elution Buffer, low salt (10 mM Tris Buffer, pH 8.5; 40 ml)

 Warm the Elution Buffer to 70°C before use.

- High Pure Filter Tubes (100 tubes)
- Collection Tubes, 2 ml (400 tubes)

### III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- PBS buffer (phosphate buffered saline)
- Isopropanol
- Lysozyme (for bacterial DNA preparations)
- Lyticase (for yeast DNA preparations)
- Disposable syringe (for mouse tail DNA preparations)


### IV. Typical nucleic acid yield from different organisms (research samples)

Starting material	Sample size	Yield (µg)
Whole blood, human	200 µl*	3 – 6 (DNA)
Buffy coat	200 µl*	20 (DNA)
Cultured cells (K562)	10 <sup>6</sup> cells	15 – 20 (DNA)
Calf thymus	25 mg	5 – 10 (DNA)
Mouse tail	0.2 – 0.5 cm (25 – 50 mg)	5 – 10 (DNA)
Yeast cells	10 <sup>8</sup> cells	10 – 13 (DNA)
Bacteria cells	10 <sup>9</sup> cells	1 – 3 (total nucleic acids)

\* 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.

### V. Protocols for preparing total nucleic acids

#### Va. Isolation of nucleic acids from 200 µl whole blood, 200 µl buffy coat, or 10<sup>4</sup> – 10<sup>5</sup> cultured mammalian cells

 If the sample is <200 µl, add PBS to make the total volume 200 µl. If the sample is >200 µl, increase all volumes proportionally in the steps below. Maximum sample volume is 300 µl.

- To a sterile 1.5 ml microcentrifuge tube:
  - ▶ Add 200 µl sample.
  - ▶ Add 200 µl Binding Buffer.
  - ▶ Add 40 µl reconstituted Proteinase K solution and mix the contents of the tube immediately.
  - ▶ Incubate tube for 10 min at 70°C.



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- 2 After the incubation, mix sample with 100 µl isopropanol.

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  - 3 To transfer the sample to a High Pure Tube:
    - ▶ Insert one High Pure Filter Tube into one Collection Tube.
    - ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube.

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  - 4 Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then centrifuge the tube assembly for 1 min at 8000 x g.

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  - 5 After centrifugation:
    - ▶ Remove the Filter Tube from the Collection Tube and discard the liquid and the Collection Tube.

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  - 6 To remove inhibitors:
    - ▶ Add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube assembly. Repeat the centrifugation step (1 min at 8000 x g) and discard flowthrough and Collection Tube.
    - ▶ Reinsert the Filter Tube in a new Collection Tube.

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  - 7 To wash the sample:
    - ▶ Add 500 µl Wash Buffer to the upper reservoir of the Filter Tube.
    - ▶ Repeat the centrifugation (as in Step 4).

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  - 8 After the first wash:
    - ▶ Repeat Step 5.
    - ▶ Repeat the wash step and centrifugation (Step 7). Discard flowthrough.
    - ▶ Spin the Filter Tube-Collection Tube assembly for 10 s at maximum speed (approx. 13,000 x g) to remove residual Wash Buffer.

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  - 9 Discard the Collection Tube and insert the Filter Tube in a clean, sterile 1.5 ml microcentrifuge tube.

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  - 10 To elute the nucleic acid:
    - ▶ Add 200 µl of prewarmed (70°C) Elution Buffer to the Filter Tube.
    - ▶ Centrifuge the tube assembly for 1 min at 8000 x g.
    - ! *To increase elution efficiency, either use more Elution Buffer or leave prewarmed Elution Buffer on glass fleece for 5 min before starting the centrifuge.*

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  - 11 The microcentrifuge tube now contains the eluted nucleic acids. You may:
    - ▶ EITHER use an aliquot of the eluted nucleic acids directly in standard or long template PCR.
    - ▶ OR store the eluted nucleic acids at 2 to 8°C for later analysis.
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**Optional RNase digestion:** If you wish to remove RNA from the template before PCR, add RNase, DNase-free (Cat. No. 11 119 915 001) to the eluted nucleic acids and incubate at 15 to 25°C or 37°C, as appropriate. For example, add 0.5 µl RNase to the nucleic acids from 10<sup>6</sup> cells and incubate 15 min at 15 to 25°C or 37°C. For nucleic acids from 10<sup>7</sup> cells, add 1.5 µl RNase and incubate 30 min at 37°C. For nucleic acids from 10<sup>8</sup> cells, add 16 µl RNase and incubate 30 min at 37°C. After treatment, the RNase can be removed from the DNA with the High Pure PCR Product Purification Kit.

**Vb. Isolation of nucleic acids from 25 – 50 mg mammalian tissue**

- 1 To a clean, sterile 1.5 ml microcentrifuge tube:
  - ▶ Add a 25 – 50 mg tissue sample.
    - ! *To increase the yield of nucleic acids, cut the sample into small pieces with a scalpel.*
  - ▶ Add 200 µl Tissue Lysis Buffer.
  - ▶ Add 40 µl reconstituted Proteinase K solution and mix the contents of the tube immediately.
  - ▶ Incubate tube for 1 h at 55°C.

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- 2 After tissue lysis:
  - ▶ Add 200 µl Binding Buffer to the tube and mix well.
  - ▶ Incubate for 10 min at 70°C.

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- 3 Add 100 µl isopropanol to the tube, then:
  - ▶ Mix the contents of the tube.
  - ▶ Draw part of the sample into a 1 ml disposable pipette tip.
    - ! *This step draws insoluble tissue segments into the pipette tip and blocks it.*
  - ▶ Withdraw and discard the pipette tip, carrying the insoluble tissue segments with it.
  - ▶ Pipette the remainder of the liquid sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
  - ▶ Follow Protocol Va above, starting at the first centrifugation step (Step 4).

**Vc. Isolation of nucleic acids from 25 – 50 mg mouse tail**

- 1 To a clean, sterile 1.5 ml microcentrifuge tube:
  - ▶ Add pieces from 0.2 – 0.5 cm (25 – 50 mg) mouse tail.
  - ▶ Add 200 µl Tissue Lysis Buffer.
  - ▶ Add 40 µl reconstituted Proteinase K solution and mix the contents of the tube immediately.
  - ▶ Incubate tube for 3 h at 55°C.

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- 2 Use a 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 above step twice.

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- 3 To the sheared sample:
  - ▶ Add 200 µl Binding Buffer.
  - ▶ Add 100 µl isopropanol and mix the contents of the tube well.
  - ▶ Centrifuge the tube 5 min at 13,000 x g.

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- 4 After the centrifugation:
  - ▶ Pipette the liquid sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
  - ▶ Follow Protocol Va above, starting at the first centrifugation step (Step 4).

**Vd. Isolation of nucleic acids from 10<sup>9</sup> bacteria or 10<sup>8</sup> yeast cells**

- 1 In a clean, sterile 1.5 ml microcentrifuge tube:
  - ▶ Collect the bacteria (10<sup>9</sup> cells) or yeast (10<sup>8</sup> cells) by low speed centrifugation (3000 x g, 5 min).
  - ▶ Resuspend the cell pellet in 200 µl PBS.
  - ▶ Does the sample contain bacteria or yeast?
    - ▶ If the sample is bacteria, go to Step 2.
    - ▶ If the sample is yeast, go to Step 3.

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- 2 To lyse bacterial cells:
  - ▶ Add 15 µl lysozyme solution (10 mg/ml in Tris-HCl, pH 8.0).
  - ▶ Incubate the tube for 15 min at 37°C.
  - ▶ Go to Step 4.

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- 3 To lyse yeast cells:
  - ▶ Add 10 µl lyticase solution (0.5 mg/ml).
  - ▶ Incubate the tube for 30 min at 30°C.
  - ▶ Go to Step 4.

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- 4 To the lysed cells from Step 2 or Step 3:
  - ▶ Add 200 µl Tissue Lysis Buffer.
  - ▶ Add 40 µl reconstituted Proteinase K solution and mix the contents of the tube immediately.
  - ▶ Incubate tube for 10 min at 70°C.

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- 5 Follow Protocol Va above, starting at the addition of isopropanol (Step 2).
  - ! *Grampositive bacteria generally require special lysis conditions for efficient DNA preparations. Data (see page 29) of cell lysis conditions for lactobacilli and for staphylococci are kindly provided by C. Bunte, J.A. Straub and C. Hertel, University of Hohenheim, Germany.*

**Ve. Isolation of nucleic acids from a formalin-fixed paraffin-embedded tissue section**

[Protocol was kindly provided by T. Fixemer, Universitäts-Kliniken des Saarlandes, Germany]

- 1 Soak the tissue section in xylene for approx. 30 min, to deparaffinize it.
  - ! *Incubation time depends on the thickness of the section.*

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- 2 Incubate the tissue section for 10 s in each of the following:
  - ▶ Absolute ethanol (dehydration)
    - ! *The section should turn white after it is transferred to ethanol.*
  - ▶ 80% ethanol
  - ▶ 60% ethanol
  - ▶ 40% ethanol
  - ▶ Distilled water (rehydration)

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- 3 To prepare the tissue sample:
  - ▶ 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, preweighed 1.5 ml microcentrifuge tube.
  - ▶ Determine the weight of the sample.



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- 4 To the tissue sample (25 – 50 mg):
- ▶ Add 200 µl Tissue Lysis Buffer.
  - ▶ Add 40 µl reconstituted Proteinase K solution and mix the contents of the tube immediately.
  - ▶ Incubate overnight at 37°C.
- ! *Optimal incubation time will depend on the type of tissue. Determine the incubation time empirically for each new tissue.*
- 
- 5 After the first incubation:
- ▶ Again, add 20 µl reconstituted Proteinase K solution.
  - ▶ Incubate at 55°C for 1 – 2 h.
- ! *After this incubation step, no crude tissue particles should be visible.*
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- 6 To the dissolved tissue sample:
- ▶ Add 200 µl Binding Buffer and mix thoroughly.
  - ▶ Incubate for 10 min at 70°C.
- 
- 7 Add 100 µl isopropanol to the tube, then:
- ▶ Mix the contents of the tube.
  - ▶ Use an automatic pipette to draw part of the sample into a 1 ml pipette tip.
- ! *Note: This treatment draws insoluble tissue segments into the pipette tip and blocks it.*
- ▶ Withdraw the pipette tip, carrying the insoluble tissue segments with it.
  - ▶ Pipette the remainder of the liquid sample into the upper reservoir of a combined Filter Tube-Collection Tube assembly.
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- 7 Follow Protocol Va, starting at the first centrifugation step (Step 4).
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## Vf. Isolation of Mycobacterial DNA

(Protocol was kindly provided by B. Leppmeier and U. Reischl, University of Regensburg, Germany)

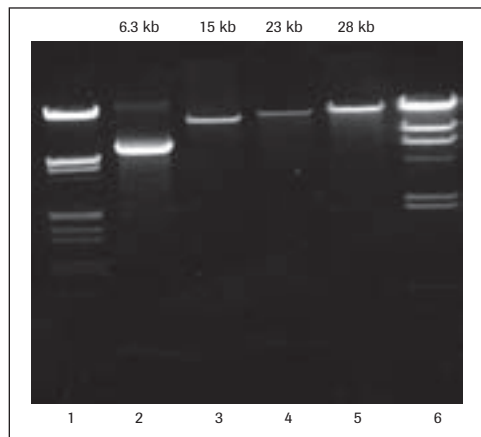
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- 1 100 µl decontaminated research sample material (respiratory samples, nonrespiratory samples or sediments from liquid Kirchner media) is centrifuged 10 min at 14,000 x g. The supernatant is aspirated by pipetting and discarded.
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- 2 Add 200 µl PBS to the pellet and resuspend by vortexing.
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- 3 Add 200 µl Binding Buffer and 40 µl Proteinase K solution to resuspend the pellet. Incubate 10 min at 70°C.
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- 4 The samples are now alternating incubated for 4 min in a boiling water bath and 2 min in liquid nitrogen. In total the samples are 6 times boiled and 5 times frozen. Then the cooled samples are collected by centrifugation for 1 min at 14,000 x g.
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- 5 Add 100 µl isopropanol and mix by vortexing.
- 
- 6 Now follow protocol Va starting with the transfer of the sample to the Filter Tube assembly (Step 3).
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## VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure PCR Template Preparation Kit, see page 99.

## Typical results with the kit

### Experiment 1



**Figure 1: Use of nucleic acids from whole human blood or cultured cells (prepared with the High Pure PCR Template Preparation Kit) for amplification of the single copy human tPA gene.** Nucleic acids were prepared from blood or cultured human K562 cells according to Protocol Va, and 250 ng of each preparation were amplified by Expand Long Template PCR. Annealing temperature was 65°C. The PCR cycling conditions and the sequence of the tPA primers were as described in the Expand Long Template PCR package insert. The amplicons obtained were:

**Lane 2:** 6.3 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 1

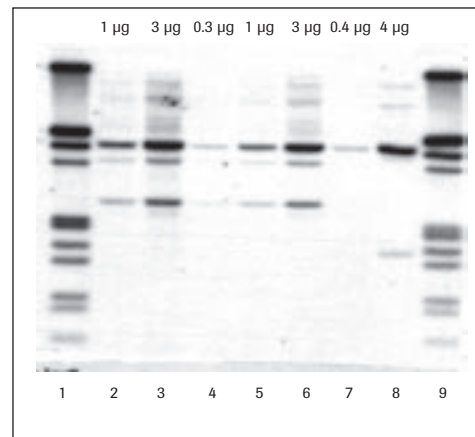
**Lane 3:** 15 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 3

**Lane 4:** 23 kb: Obtained from blood, amplified in Expand Long Template PCR buffer 3

**Lane 5:** 28 kb: Obtained from K562 cells, amplified with the Expand 20 kbPLUS System

**Result:** All preparations yielded a distinct specific band of the expected fragment length even as large as 28 kb from genomic DNA prepared with the High Pure PCR Template Preparation Kit.

### Experiment 2



**Figure 2: Use of nucleic acids from mammalian cells or tissue (prepared with the High Pure PCR Template Preparation Kit) for detection of the actin gene on a Southern blot.** Nucleic acids were purified from cultured mammalian cells ( $10^8$  K562 cells) by Protocol Va and from calf thymus tissue (25 mg) by Protocol Vb. Aliquots of the preparations were digested with Eco RI, purified (see below), separated electrophoretically, and transferred to a membrane by Southern blotting. The blot was hybridized to a DIG-labeled  $\beta$ -actin antisense RNA probe according to standard procedures. The hybridized bands were detected with an alkaline phosphatase-labeled anti-DIG antibody and visualized chemiluminescently with CSPD substrate. The blot was exposed to X-ray film for 1 h.

**Lanes 1, 9:** DNA Molecular Weight Marker III

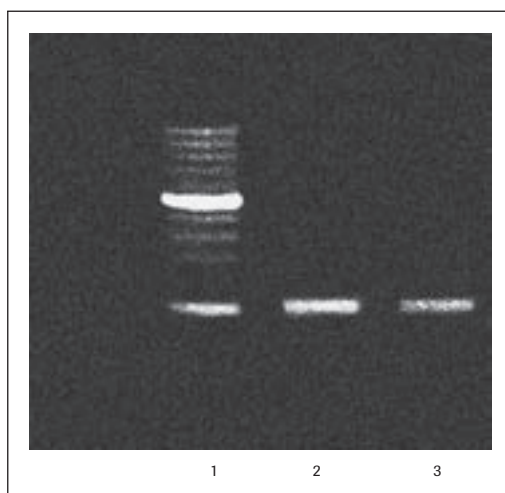
**Lanes 2, 3:** 1  $\mu$ g and 3  $\mu$ g samples of K562 genomic DNA, purified with the High Pure PCR Product Purification Kit after Eco RI digestion

**Lanes 4 – 6:** 0.3  $\mu$ g, 1  $\mu$ g, and 3  $\mu$ g samples of K562 genomic DNA, purified by traditional methods (phenol/chloroform extraction, ethanol precipitation) after Eco RI digestion

**Lanes 7, 8:** 0.4  $\mu$ g and 4  $\mu$ g samples of calf thymus genomic DNA, purified by traditional methods (phenol/chloroform extraction, ethanol precipitation) after Eco RI digestion

**Result:** All genomic DNA samples isolated with the High Pure PCR Template Preparation Kit were readily and completely cut by Eco RI, were suitable for Southern blot analysis, and contained the expected small and large fragments of the actin gene.

### Experiment 3



**Figure 3: Use of nucleic acids from paraffin-embedded tissue (prepared with the High Pure PCR Template Preparation Kit) for amplification of the human androgen receptor gene.**

Nucleic acids were purified from formalin-fixed, paraffin-embedded samples of human prostate tissue by Protocol Ve. An aliquot (10  $\mu$ l) of each nucleic acid product was used as template for the Expand High Fidelity PCR System (100  $\mu$ l reaction mixture). The primers were derived from exon 7 of the androgen receptor gene. Aliquots (20  $\mu$ l) of each amplicon were separated electrophoretically and the gel was stained with ethidium bromide.

**Lane 1:** Molecular Weight Marker VIII

**Lane 2:** Androgen receptor amplicon from one tissue section

**Lane 3:** Androgen receptor amplicon from half a tissue section

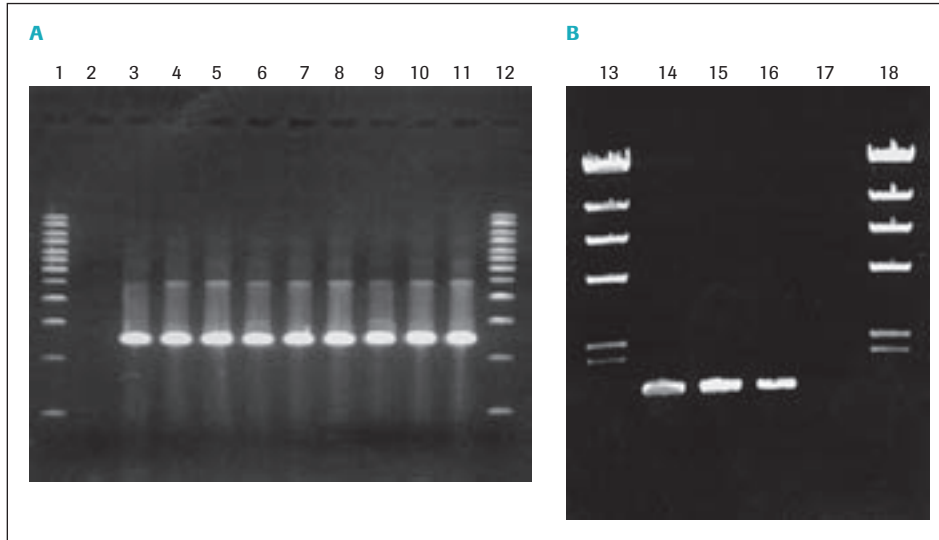
**Result:** The 263 bp androgen receptor amplicon was clearly visible in both tissue samples.

### Experiment 4

	Incubation period		
	15 min	30 min	60 min
<i>L. casei</i>			
Without Mu	15.2 (40.0 %)	20.2 (53.2 %)	21.8 (57.4 %)
0.25 U/ $\mu$ l Mu	15.3 (40.3 %)	24.6 (64.7 %)	24.0 (63.2 %)
0.5 U/ $\mu$ l Mu	16.4 (43.2 %)	38.0 (100 %)	30.4 (80.0 %)
<i>L. curvatus</i>			
Without Mu	9.2 (10.6 %)	14.7 (17.0 %)	26.1 (30.2 %)
0.25 U/ $\mu$ l Mu	25.9 (29.9 %)	61.3 (70.9 %)	70.7 (81.7 %)
0.5 U/ $\mu$ l Mu	46.0 (53.2 %)	86.5 (100 %)	131.1 (151.6 %)
<i>L. sakei</i>			
Without Mu	13.5 (11.4 %)	16.6 (14.0 %)	52.9 (44.7 %)
0.25 U/ $\mu$ l Mu	35.0 (29.6 %)	71.8 (60.6 %)	168.2 (142.1 %)
0.5 U/ $\mu$ l Mu	79.3 (67.0 %)	118.4 (100 %)	196.1 (165.6 %)
<i>S. aureus</i>			
Without Ly	10.3 (10.8 %)	30.5 (31.9 %)	64.2 (67.2 %)
10 $\mu$ g/ml Ly	16.5 (17.3 %)	95.0 (99.5 %)	105.2 (110.2 %)
25 $\mu$ g/ml Ly	16.2 (17.0 %)	95.5 (100 %)	106.7 (111.7 %)

**Table 1: Use special lysis conditions for gram-positive bacteria to achieve high DNA concentrations ( $\mu$ g/ml) in eluate.** The Lysis Buffer contained 10 mg/ml lysozyme for lactobacilli and 1 mg/ml lysozyme for staphylococci together with the amounts of mutanolysin (MU) or lysostaphin (Ly) shown in the first column. The percentages refer to the DNA yield obtained with the highest mutanolysin or lysostaphin concentration and an incubation period of 30 min.

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**Figure 4: Agarose gel electrophoresis of the PCR products for *S. aureus* (A) and for various lactobacilli (B)**

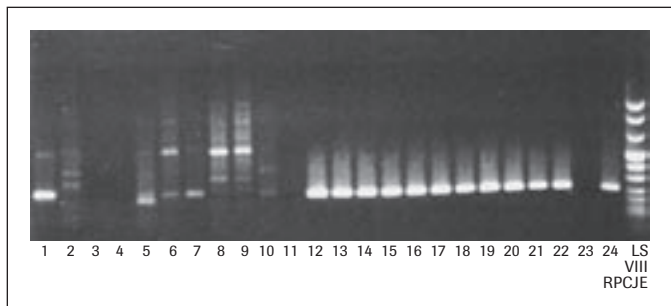
**A:**

**Lanes 1 and 12:** Molecular Weight Marker 1 kb ladder  
**Lane 2:** Negative control (without DNA)  
**Lanes 3 – 5:** *S. aureus*; lysis without lysostapin, incubation periods 15, 30 and 60 min  
**Lanes 6 – 8:** *S. aureus*; lysi with 10 µg/ml lysostapin, incubation periods 15, 30 and 60 min  
**Lanes 9 – 11:** *S. aureus*; lysis with 25 µg/ml lysostapin, incubation periods 15, 30 and 60 min

**B:**

**Lanes 13 and 18:** Marker I DNA Hind III;  
**Lane 14:** *L. casei*  
**Lane 15:** *L. curvatus*  
**Lane 16:** *L. sakei*  
**Lane 17:** Negative control (without DNA)

## Experiment 5



**Figure 5: Gel electrophoresis of PCR products and controls.**

**Lane 1:** Smear +++; Specimen sputum; known *M. tuberculosis* infection  
**Lane 2:** Smear -; Specimen sputum  
**Lane 3:** Smear -; Specimen feces  
**Lane 4:** Smear -; Specimen feces; double because of different wash step  
**Lane 5:** Smear +/-; Specimen sputum  
**Lane 6:** Smear +; Specimen BAL  
**Lane 7:** Smear ++; Specimen sputum; COBAS Amplicor MTB +  
**Lane 8:** Smear ++; Specimen sputum; known *M. avium* infection

**Lane 9:** Smear ++; Specimen sputum; known *M. avium* infection  
**Lane 10:** Smear +; Specimen sputum  
**Lane 11:** Smear +; Specimen liquid Kirchner media sediment; Smear: acid-fast, rod-shaped  
**Lanes 12 – 22:** Internal controls of samples of lane 1 – 11  
**Lane 23:** Negative control  
**Lane 24:** Positive control *M. tuberculosis* H37  
**Lane 25:** Molecular Weight Marker VIII (Roche Applied Science)

Microbacterial DNA was isolated from clinical research specimen or sediments of liquid cultures by the High Pure PCR Template Preparation Kit. The eluted DNA was analyzed by amplification detection in *M. tuberculosis*-specific PCR (IS6110 target). To control the successful amplification an agarose gel electrophoresis was performed.

**Results:** All internal controls (100 pg *M. tuberculosis* H37 DNA) are positive (lanes 12 – 22, 242 bp amplification product). This indicates the absence of PCR inhibitors within the DNA preparation. Samples of lanes 1, 5, 6, 7, 10 are assessed as positive which is in accordance with the corresponding smear results.

## LightCycler® PCR sample preparation

The High Pure PCR Template Preparation Kit has been evaluated for LightCycler® PCR sample preparation with whole blood and cultured cells as sample material. Amplification has been performed in LightCycler® capillaries using SYBR Green and Hybridization Probes as the detection formats. 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 concentration range [ng/μl]	Eluate use in LightCycler® PCR (μl)
Whole blood human*	1 – 50 μl **	0.5 – 25	1 – 5 [0.5 ng – 125 ng]
Cultured cells, K562*	100 – 10 <sup>5</sup> cells	0.01 – 10	1 – 5 [0.01 – 50 ng]

\* Research samples

\*\* Yield 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 LightCycler® sample preparation 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		HybProbes	
		Min	Max	Min	Max
cyclophilin A	blood	0.005 μl <sup>a)</sup>	100 μl	0.005 μl <sup>a)</sup>	100 μl
or β-globin	cells	1 <sup>a)</sup>	10 <sup>4</sup>	1 <sup>a)</sup>	10 <sup>5</sup>

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

a) The used quantities of eluate correspond to these calculated amounts.

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## References

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