For general laboratory use.

High Pure PCR Template Preparation Kit

Version: 28
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For isolation of nucleic acids for PCR and Southern blotting.

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

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Content
1	white	High Pure PCR Template Preparation Kit, Tissue Lysis Buffer	Contains 4 M urea, 200 mM Tris, 20 mM NaCl, 200 mM EDTA, pH 7.4 (+25°C).	1 bottle, 20 mL
2	green	High Pure PCR Template Preparation Kit, Binding Buffer	Contains 6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, 20% polidocanol (w/v).	1 bottle, 20 mL
3	pink	High Pure PCR Template Preparation Kit, Proteinase K	▪ Lyophilized ▪ Recombinant, PCR grade ▪ For sample lysis and inactivation of endogenous DNase.	1 vial
4a	black	High Pure PCR Template Preparation Kit, Inhibitor Removal Buffer	Contains 5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6 (+25°C); final concentration after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 33 mL
4	blue	High Pure PCR Template Preparation Kit, Wash Buffer	Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C); final concentration after addition of ethanol. *See Section* **Working Solution** *for information on preparing the solution.*	1 bottle, 20 mL
5	colorless	High Pure PCR Template Preparation Kit, Elution Buffer	Contains 10 mM Tris-HCl, pH 8.5 (+25°C).	1 bottle, 40 mL
6	–	High Pure PCR Template Preparation Kit, High Pure Filter Tubes	For use of up to 700 µL sample volume.	2 bags, 50 polypropylene filter tubes with two layers of glass fiber fleece each
7	–	High Pure PCR Template Preparation Kit, Collection Tubes	For nucleic acid isolation.	8 bags, 50 polypropylene tubes, 2 mL each

⚠ ***All solutions are clear, except Bottle 1 Binding Buffer which can be clear to slightly turbid, and colorless to a slightly yellowish viscous solution. The buffers should not be used if precipitates are present. Warm the solutions at +15 to +25°C or in a +37°C water bath until the precipitates are 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)

When stored at +15 to +25°C, the kit is stable through the expiry date printed on the label.

Vial / bottle	Cap	Label	Storage
1	white	Tissue Lysis Buffer	Store at +15 to +25°C. ⚠ ***Storage at +2 to +8°C or −15 to −25°C will adversely impact nucleic acid isolation due to the formation of precipitates in the solutions and may result in reduced binding efficiency.***
2	green	Binding Buffer	
3	pink	Proteinase K	
4a	black	Inhibitor Removal Buffer	
4	blue	Wash Buffer	
5	colorless	Elution Buffer	
6	–	High Pure Filter Tubes	Store at +15 to +25°C.
7	–	Collection Tubes	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents
- Absolute ethanol
- Absolute isopropanol
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Sterile microcentrifuge tubes, 1.5 mL

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 High Pure PCR Template Preparation Kit isolates 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

Assay Time

	Whole blood and cell culture	Tissue
Total time	Approximately 20 minutes.	Approximately 2 hours.
Hands-on time	Approximately 12 minutes.	Approximately 30 minutes.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolation of nucleic acids from a variety of 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 and precautions

⚠ ***Binding Buffer and Inhibitor Removal Buffer contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
- Never store or use the Binding Buffer and Inhibitor Removal Buffer near human or animal food.
- Avoid contact of the Binding Buffer and Inhibitor Removal Buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash the affected area with a large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.
- Do not use any modified ethanol.
- Do not pool reagents from different lots or from different bottles of the same lot.
- 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 and Inhibitor Removal Buffer to mix with sodium hypochlorite 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.
- Use only calibrated pipettes.
- 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 pipettes and nuclease-free pipette 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 setup. Sample preparation, PCR/RT-PCR setup, 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 documentation.roche.com, or upon request from the local Roche office.

Working Solution

Prepare the following working solutions:

Content	Reconstitution / preparation	Storage and stability	For use in...
Proteinase K (Vial 3)	Pipette 4.5 mL of double-distilled water into the glass vial containing lyophilized Proteinase K. ▪ Add the rubber stopper and invert the vial until all the lyophilizate, including any lyophilizate stuck to the rubber stopper, is completely dissolved. ▪ Aliquot the reconstituted enzyme, labeling each aliquot with the date of reconstitution.	▪ Store at −15 to −25°C. ▪ Stable for 12 months.	Protocol, Step 1: Sample lysis and DNA binding
Inhibitor Removal Buffer (Bottle 4a)	Add 20 mL absolute ethanol to Inhibitor Removal Buffer and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable through the expiry date printed on kit label.	Protocol, Step 1: Washing and elution
Wash Buffer (Bottle 4)	Add 80 mL absolute ethanol to Wash Buffer and mix well. ⚠ ***Label and date bottle after adding ethanol.***	▪ Store at +15 to +25°C. ▪ Stable through expiry date printed on kit label.	Protocol, Steps 2 and 3: Washing and elution

2.2. Protocols

Experimental overview

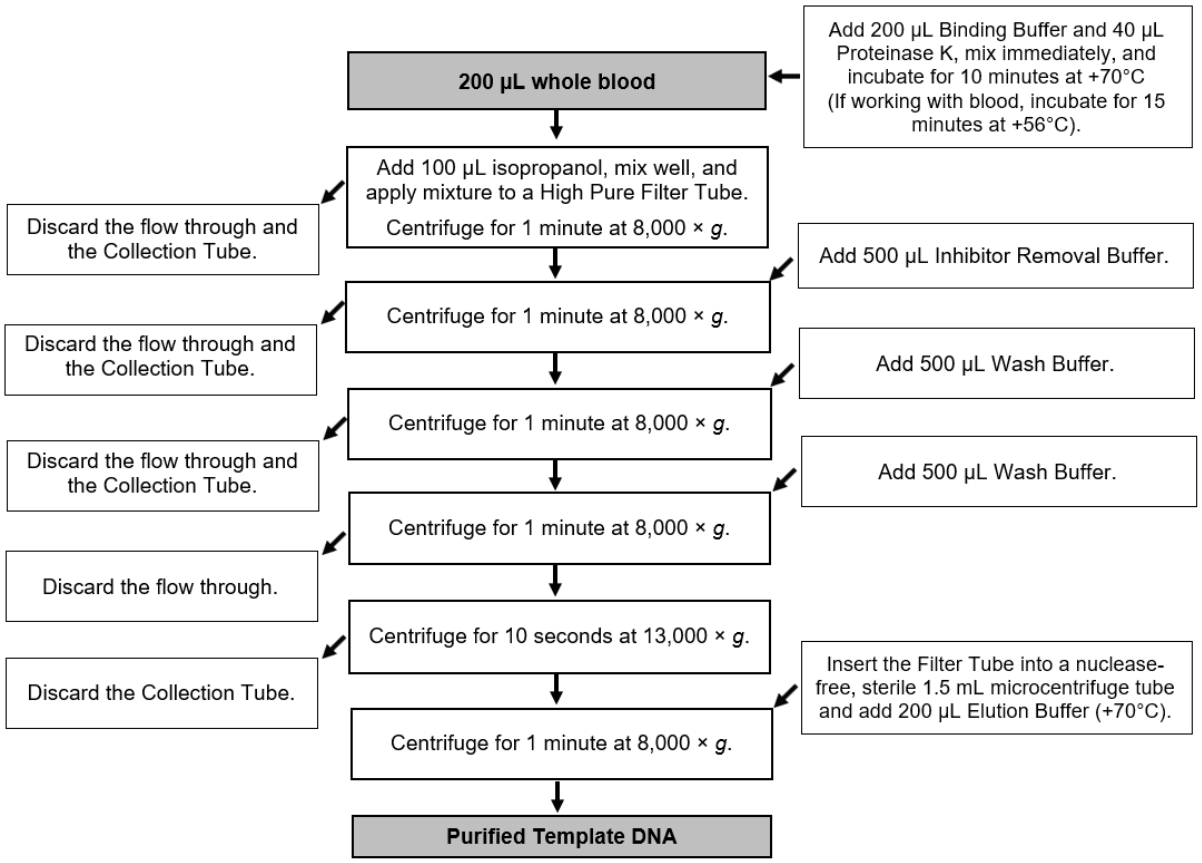

Fig.1: Isolation of nucleic acids from mammalian whole blood, buffy coat, or cultured cells.

Adjustment of sample volume

When your sample volume is less than 200 µL, increase the sample volume with PBS according to the following table:

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 reagents volumes accordingly.

Isolation of nucleic acids from 200 µL mammalian whole blood, 200 µL buffy coat, or 10^4 to 10^6 cultured cells

See Section **Working Solution** *for additional information on preparing solutions.*

Sample lysis and DNA binding

For isolation of nucleic acids, use prewarmed Elution Buffer (+70°C).

1. To a nuclease-free 1.5 mL microcentrifuge tube, add:
   – 200 µL of sample material.
   – 200 µL Binding Buffer.
   – 40 µL reconstituted Proteinase K.
   – Mix immediately and incubate at +70°C for 10 minutes.

   If working with blood, incubate for 15 minutes at +56°C.

2. Add 100 µL isopropanol and mix well.

3. Insert one High Pure Filter Tube into one Collection Tube.
   – Pipette the remainder of the sample into the upper reservoir of the Filter Tube (maximum 700 µL).
   – Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   – Centrifuge 1 minute at 8,000 × *g*.

4. Proceed to Section **Protocol for washing and elution**.

Isolation of nucleic acids from 25 to 50 mg mammalian tissue

Sample lysis and DNA binding

For isolation of nucleic acids, use prewarmed Elution Buffer (+70°C).

1. To a nuclease-free 1.5 mL microcentrifuge tube, add:
   – 25 to 50 mg of sample material.
   – 200 µL Tissue Lysis Buffer.
   – 40 µL reconstituted Proteinase K.
   – Mix immediately and incubate for 1 hour 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.

2. Add 200 µL Binding Buffer.
   – Mix immediately and incubate for 10 minutes at +70°C.

3. Add 100 µL isopropanol and mix well.
   – Draw a fraction of the sample into a 1 mL disposable pipette tip.

   This procedure 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. Insert one High Pure Filter Tube into one Collection Tube.
   – Pipette the remainder of the sample into the upper reservoir of the Filter Tube.

5. Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   – Centrifuge 1 minute at 8,000 × *g*.

6. Proceed to Section **Protocol for washing and elution**.

Isolation of nucleic acids from 25 to 50 mg mouse tail tissue

Sample lysis and DNA binding

⚠ ***For isolation of nucleic acids, use prewarmed Elution Buffer (+70°C).***

1. To a nuclease-free 1.5 mL microcentrifuge tube, add:
   – 0.2 to 0.5 cm (25 to 50 mg) mouse tail.
   – 200 µL Tissue Lysis Buffer.
   – 40 µL reconstituted Proteinase K.
   – Mix immediately and incubate for 3 hours 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 and 100 µL isopropanol and mix well.
   – Centrifuge 5 minutes at 13,000 × *g*.

4. Insert one High Pure Filter Tube into one Collection Tube..
   – Pipette the remainder of the sample into the upper reservoir of the Filter Tube.

5. Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
   – Centrifuge 1 minute at 8,000 × *g*.

6. Proceed to Section **Protocol for washing and elution**.

Isolation of nucleic acids from bacteria or yeast cells

ⓘ *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

⚠ ***For isolation of nucleic acids, use prewarmed Elution Buffer (+70°C).***

1. To a nuclease-free 1.5 mL microcentrifuge tube, add:
   – 200 µL bacteria or yeast cells.
   – Centrifuge for 5 minutes at 3,000 × *g*.
   – Resuspend cell pellet in 200 µL PBS.

2. For bacteria:
   – Add 5 µL lysozyme (10 mg/mL in 10 mM Tris-HCl, pH 8.0) and incubate 15 minutes at +37°C.
   For Yeast:
   – Add and 10 µL lyticase (0.5 mg/mL) and incubate 30 minutes at +37°C.

3. Add 200 µL Binding Buffer and 40 µL reconstituted Proteinase K.
   – Mix immediately and incubate for 10 minutes at +70°C.
   – Add 100 µL isopropanol and mix well.

4. Insert one High Pure Filter Tube into one Collection Tube.
   – Pipette the remainder of the sample into the upper reservoir of the Filter Tube.

5 Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
– Centrifuge 1 minute at 8,000 × *g*.

6 Proceed to Section **Protocol for washing and elution**.

Isolation of nucleic acids from 25 to 50 mg formalin-fixed, paraffin-embedded tissue sections

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

Sample lysis and DNA binding

⚠ ***For isolation of nucleic acids, use prewarmed Elution Buffer (+70°C).***

1 Deparaffinize the tissue section in xylene for approximately 30 minutes.

Incubation time depends on the thickness of the section.

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

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 25 to 50 mg tissue sample, add:
– 200 µL Tissue Lysis Buffer.
– 40 µL reconstituted Proteinase K.
– Mix and incubate overnight at +37°C.

5 Add 20 µL reconstituted Proteinase K.
– Incubate for 1 to 2 hours at +55°C.

After this incubation, no tissue particles should be visible.

6 Add 200 µL Binding Buffer, mix thoroughly, and incubate 10 minutes 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 and discard the pipette, thereby removing the insoluble tissue particles.

8 Insert one High Pure Filter Tube into one Collection Tube.
– Pipette the remainder of the sample into the upper reservoir of the Filter Tube.

9 Insert the entire High Pure Filter Tube assembly into a standard tabletop centrifuge.
– Centrifuge 1 minute at 8,000 × *g*.

10 Proceed to Section **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.
   – Insert the Filter Tube into a new Collection Tube.
   – Add 500 µL Inhibitor Removal Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge 1 minute at 8,000 × *g*.

2. Remove the Filter Tube from the Collection Tube; discard the flow through liquid, and the Collection Tube.
   – Insert the Filter Tube into a new Collection Tube.
   – Add 500 µL Wash Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge 1 minute 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.
   – Insert the Filter Tube into a new Collection Tube.
   – Add 500 µL Wash Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge 1 minute at 8,000 × *g* and discard the flow through.

4. Leave the Filter Tube-Collection Tube assembly in the centrifuge and spin it for 10 seconds at maximum speed (approximately 13,000 × *g*) to remove any residual Wash Buffer.

   i *This extra centrifugation step ensures removal of residual Wash Buffer.*

5. Discard the Collection Tube and insert the Filter Tube into a nuclease-free, sterile 1.5 mL microcentrifuge tube.

6. To elute the nucleic acids:
   – Add 200 µL prewarmed Elution Buffer to the upper reservoir of the Filter Tube.
   – Centrifuge the tube assembly for 1 minute at 8,000 × *g*.

7. The microcentrifuge tube now contains the eluted nucleic acids.
   – Use the eluted nucleic acids directly or store the eluted nucleic acid at +2 to +8°C or −15 to −25°C for later analysis.
   – To add an RNase digestion, see 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 minutes at +15 to +25°C or +37°C.

3. Results

Purity

Purified nucleic acids are free of other cellular components and DNA polymerase inhibitors.
RNA can be removed from purified nucleic acid with an optional RNase digestion.

Sensitivity

The High Pure PCR Template Preparation Kit has been evaluated using the LightCycler® 480 Instrument II for whole human blood and cultured K-562 cells. Amplification was performed in the LightCycler® 480 Instrument II using the Hydrolysis/UPL Probe detection format.
The following tables indicates the range of sample volume and concentration, as well as expected results. Each Isolation was performed with 12 replicates followed by duplicate measurements on the LightCycler® 480 Instrument II to assess the mean Cp.

Sample	WBC/cell count	Mean Cp	SD	Input volume	Eluate volume	Target	Instrument
Frozen human whole blood	4.5×10^3 cells/µL	26.06	0.04	200 µL	5 µL	Betaglobin	LightCycler® 480 Instrument II
	9.1×10^3 cells/µL	24.57	0.13				
Frozen cultured cells, K-562	10^4 cells/mL	28.06	0.35	In 200 µL PBS			
	10^6 cells/mL	23.17	0.51				

Expected yield

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

Sample	Amount	Input volume	Yield [µg][1] Total nucleic acids
Frozen human whole blood	$5 – 10 \times 10^3$ cells/µL	200 µL	8 – 12[2]
Frozen buffy coat	–	200 µL	4 – 8[2]
Mouse tail	50 mg	In 200 µL TLB	35 – 40
FFPET	35 mg (50 µm)	In 200 µL TLB	7 – 12
Frozen cultured cells, K-562	10^6 cells/mL	In 200 µL PBS	7 – 10

[1] Yields from the different sample types may vary depending on the storage conditions.
[2] Yields may vary between different blood donors due to varying numbers of leukocytes.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity.	Kit stored under suboptimal conditions.	Store kit at +15 to +25°C.
	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 to avoid contamination.
		Store reconstituted reagents at either +2 to +8°C or −15 to −25°C, as indicated in the Instructions for Use.
	Ethanol not added to Wash Buffer and Inhibitor Removal Buffer.	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always label Wash Buffer and Inhibitor Removal Buffer bottles to indicate whether ethanol has been added.
	Reagents and samples not completely mixed.	Always mix the sample tube well 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.
Low yield.	Incomplete Proteinase K dissolution.	Be sure to dissolve the lyophilized Proteinase K completely. *See Section,* **Working Solution** *for additional information.*
	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/purity from buffy coat or high Cp values.	Incomplete lysis, overloading the silica membrane.	1:1 dilution of sample material with PBS.
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: ▪ Incubate tissue with Proteinase K overnight. ▪ Incubate with Proteinase K for 3 to 4 hours, then add a fresh aliquot of Proteinase K (30 µL) and incubate another 1 to 2 hours. ⚠ ***To accommodate increased volume of sample and enzyme, use 230 µL Binding Buffer, instead of the 200 µL indicated in Step 2, see Section Protocols, 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 Instructions 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 stored at −15 to −25°C from the time of harvest until lysis.
		Use smaller pieces of tissue (20 to 40 mg) or homogenize tissue sample.
Eluate from blood is still slightly colored.	Incomplete wash.	▪ Wash Filter Tube until flow through is colorless. ▪ 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. ▪ Follow the same procedure, starting with the application of the sample to the High Pure Filter Tube, see Section **Isolation protocol for preparing nucleic acids from 200 µL mammalian whole blood, 200 µL buffy coat, or 10^4 to 10^6 cultured cells, Step 4**. ⚠ ***Omit Proteinase K digestion and +70°C incubation.***

5. Additional Information on this Product

5.1. Test Principle

How this product works

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 nucleic acid 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 nucleic acid 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

For lot-specific certificates of analysis, see Section **Contact and Support**.

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

Layout changes.
Editorial changes.
Change of the constituent of Binding buffer in the Content Chapter.
Removed information related to the REACH Annex XIV.
Adapted the Result Chapter to show data derived when the new Binding Buffer is used.
Update of the Troubleshooting Chapter.

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		
RNase, DNase-free	500 µg, 1 mL	11 119 915 001
Lysozyme	10 g, *Not available in US*	10 837 059 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 L	11 666 789 001
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
Expand Long Template PCR System	150 U, 1 x 150 U 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
Poly(A)	100 mg	10 108 626 001

6.4. Trademarks

LIGHTCYCLER is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For additional documentation such as certificates and safety data sheets, please visit:
documentation.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

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.

Visit **documentation.roche.com**, to download or request copies of the following Materials:
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- Safety Data Sheets
- Certificates of Analysis
- Information Material

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