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High Pure PCR Cleanup Micro Kit

Version 05
Content version July 2017

For purification of PCR products

Cat. No. 04 983 955 001 Kit for up to 50 purifications
Cat. No. 04 983 912 001 Kit for up to 200 purifications

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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1. What this Product Does

Number of Tests

The kit is designed to perform up to 50 or up to 200 purifications (depending on pack size).

Both pack sizes of the kit contain the same components; they differ only in how much of each component they contain.

Kit Contents

All solutions are clear. If any solution contains a precipitate, warm the solution at +15°C to +25°C or in a 37°C waterbath to dissolve the precipitate.

Do not use vessels or pipettes containing polystyrene (PS) when working with the Binding Enhancer (vial 2).

Vial/Cap	Label	Contents / Function A: Cat. No. 04 983 955 001 B: Cat. No. 04 983 912 001
1 green	Binding Buffer	**A**: 20 ml **B**: 80 ml • for binding nucleic acids
2 colorless	Binding Enhancer	**A**: 20 ml **B**: 2 × 20 ml • for adjustment of stringency of binding reaction
3 blue	Wash Buffer	**A**: 10 ml, add 40 ml absolute ethanol **B**: 2 × 20 ml, add 80 ml absolute ethanol to each vial wash buffer
4 colorless	Elution Buffer	**A/B:** 40 ml 10 mM Tris-HCl, pH 8.5 (25°C)
5	High Pure Micro Filter Tubes	**A:** 5 bags **B**: 20 bags • each with 10 micro columns; for processing up to 500 µl sample volume per column
6	Collection Tubes	**A**: 1 bag **B:** 4 bags • each with 50 polypropylene tubes (2 ml) per bag

Storage and Stability

Store the High Pure PCR Cleanup Micro Kit components at +15 to +25°C. Kit components are guaranteed to be stable through the expiration date printed on the label.

Improper storage of the kit, *e.g.* at either +2 to +8°C (refrigerator) or -15 to -25°C (freezer), may lead to formation of salt precipitates in the buffers,

which will adversely affect the performance of the kit. High Pure isolation kits are always shipped at +15 to +25°C, and should be stored at that temperature.

Additional Equipment and Reagents Required

The following additional reagents and equipment are required for cleaning up DNA fragments from **solutions:**

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

The following additional reagents and equipment are required for cleaning up DNA fragments from **agarose slices**:

- Absolute ethanol
- Agarose
- TAE buffer (40 mM Tris-acetate, 1 mM EDTA) pH 8.4 or
- TBE buffer (89 mM Tris-borate, 2 mM EDTA) pH 8.4
- Electrophoresis equipment
- Sterile scalpel
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

Application

The kit is designed to efficiently isolate PCR products from amplification reactions. The kit removes primers, salts, unincorporated nucleotides, and thermostable DNA polymerase, all of which may inhibit subsequent enzymatic reactions (*e.g.* labeling, sequencing or cloning of the PCR products).

In addition, the kit can isolate nucleic acids form other reactions (*e.g.* restriciton endonuclease digests, polishing reactions, or nonradioacitive labeling reactions). The kit is also recommended for purifying cDNA. It can also be used to concentrate dilute nucleic acid solutions and recover DNA from agarose slices.

Since the binding enhancer is added to the reaction separately, the user can adjust the stringency of the purification procedure. Increasing the amount of binding enhancer used in the binding step (up to maximum of 40%) causes DNA molecules of lower molecular weight to be bound and purified more efficiently. If less binding enhancer is used, small DNA fragments are more likely to be lost during the purification. In some cases elimination of small DNA fragments may be useful, especially when PCR products that contain primer-dimer artifacts are purified for downstream analysis. For further information please refer to section 2.2 of this manual.

Preparation Time

The entire High Pure PCR Cleanup Micro Kit method takes approx. 10 min.

2. How to Use this Product

2.1 Before You Begin

Precautions

⚠ Binding Buffer contain guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling. Specifically:

- Do not let Binding Buffer touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.
- Never store or use the Binding Buffer near human or animal food.

Sample Material

Samples (up to 100 µl each) could contain:

- Amplified DNA products that are between 50 bp and 5 kb long
- Modified DNA fragments [*e.g.*, DNA processed with restriction enzymes (1), T4 polymerase or other enzymes (2,3)] that are between 50 bp and 5 kb long
- Hapten-labeled (*e.g.,* DIG-labeled) or fluorescently labeled DNA fragments
- RNA from *in vitro* transcription reactions
- First and second strand cDNA (4)
- Samples (up to 100 mg) of agarose gel slices (5)

Preparation on Working Solutions

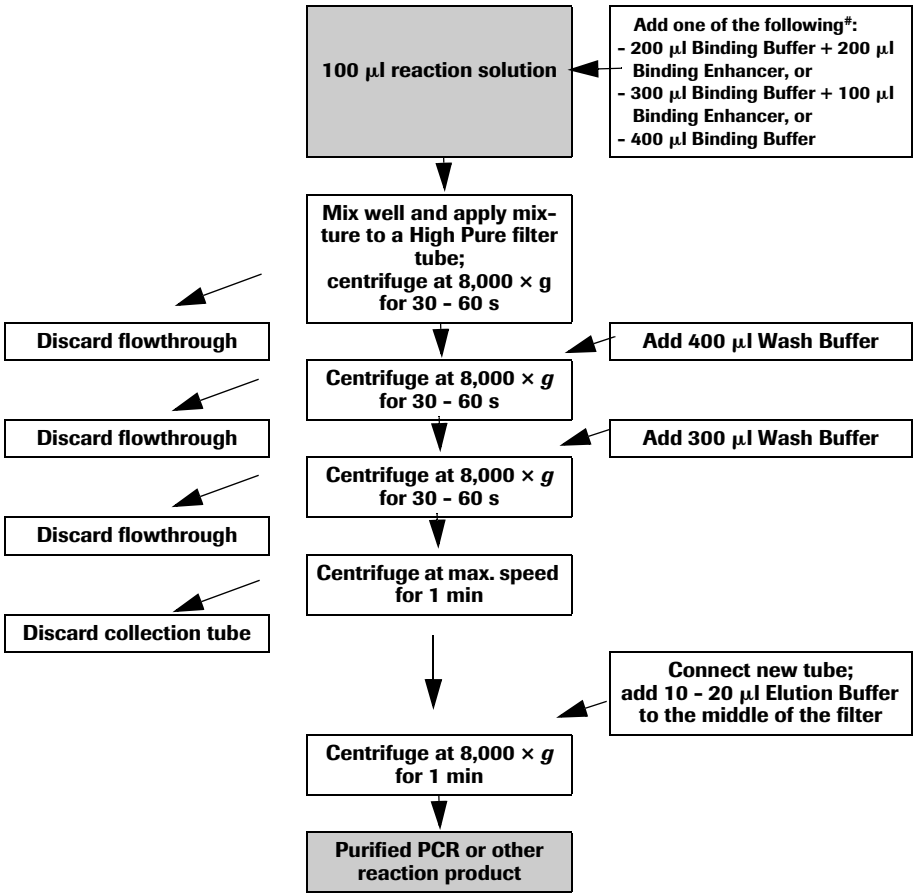
Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution

Content	Preparation	Storage/ Stability	For use in
Wash Buffer (Vial 3, blue cap)	**A:** Add 40 ml absolute ethanol **B:** Add 80 ml absolute ethanol to each vial Wash Buffer. ✎ Label and date bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through expiration date printed on kit label.	PCR product purification; removal of nucleotides, primers, salts and proteins.

2.2 Application Selection Guide

For 100 µl liquid sample or 100 mg agarose gel slice		200 µl Binding Buffer + 200 µl Binding Enhancer (40%)	300 µl Binding Buffer + 100 µl Binding Enhancer (20%)	400 µl Binding Buffer
Purification of liquid sample, 100 µl	Labeling or other reaction products 100 bp to 5 kb	+		
	PCR products 50 bp to 5 kb	+		
	DNA fragments for sequencing			+
Purification from Agarose Gel, 100 mg	DNA fragments 100 bp to 5 kb		+	
Removal of low molecular DNA	Primer up to 25 bases		+	
	Primer-Dimer up to 70 bp			+

2.3 Experimental overview



Please select from the Application Selection Guide (section 2.2).

2.4 Purification of PCR Products from Solution

Use the following procedure to remove low molecular weight DNA up to 70 bp from PCR or other reactions. For other applications please refer to section 2.2 Application Selection Guide.

To process a larger sample (>100 µl), divide it into several 100 µl aliquots and process each as a separate sample.

Make sure that your sample is in a tube that can hold more than 500 µl. If the sample is > 100 µl, it should be in a 1.5 ml tube.

1. • After the PCR is finished, adjust total volume of each sample to 100 µl by adding double dist. water.
   • To each 100 µl PCR sample, add:
   • 400 µl Binding Buffer
   • Mix sample well by vortexing (*e.g.*, vortex twice, for 4 s each).
   • Centrifuge the mixture briefly.

2. • Insert one High Pure Filter Tube into one Collection Tube.
   • Using a pipette, transfer the sample from step 1 to the upper reservoir of the Filter Tube.
   • Centrifuge 30-60 s at 8000 × *g* in a standard table top centrifuge at +15 to +25°C.

3. • Disconnect the Filter Tube, and discard the flowthrough solution.
   • Reconnect the Filter Tube to the same Collection Tube.

4. • Add 400 µl Wash Buffer to the upper reservoir.
   • Centrifuge 30-60 s at 8000 × *g* (as above).

5. • Discard the flowthrough solution.
   • Reconnect the Filter Tube to the same Collection Tube.
   • Add 300 µl Wash Buffer.
   • Centrifuge 30-60 s at 8000 × *g* (as above).

   ⚠ This second (300 µl) wash step ensures optimal purity.

6. • Discard the flowthrough solution.
   • Reconnect the Filter Tube to the same Collection Tube.
   • Centrifuge 1 min at maximum speed.
   • Discard the flowthrough solution and the Collection Tube.
   • Connect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

   ⚠ This step ensures complete removal of Wash Buffer.

7. • Add 10 - 20 µl Elution Buffer to the center of the Filter Tube.
   • Centrifuge 1 min at 8,000 × *g*.

   ⚠ Do not use water for elution, since alkaline pH is required for optimal yield.

8. The microcentrifuge tube now contains the purified DNA.

   ⚠ If you plan to determine the A_{260} of the eluted DNA, first centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers, which may interfere with the absorbance measurement. Use an aliquot of the supernatant to determine concentration

   Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or -15 to -25°C for later analysis.

2.5 Purification of DNA Fragments from Agarose Gel

Use the following procedure to purify DNA from a 100 mg agarose gel slice.

1. Isolate DNA band of interest via agarose gel electrophoresis as follows:
   - Load PCR product mixture on a 0.8 - 2% agarose gel.
   - Use 1 × TAE* or 1 × TBE* as running buffer.
   - Electrophorese until DNA band of interest is separated from adjacent contaminating fragments.

2. Identify bands by staining gel with ethidium bromide or SYBR Green I Nucleic Acid Gel Stain*.

   ⚠ Wear gloves; ethidium bromide is a potent carcinogen.

3. Cut desired DNA band from gel using a scalpel or razor blade that has been sterilized with ethanol.

   ⚠ Minimize volume of slice by placing gel on a UV light box (to make the DNA visible) and cutting the smallest possible gel slice that contains the desired DNA.

4. - Preweigh an empty, sterile 1.5 ml microcentrifuge tube.
   - Place excised agarose gel slice in the sterile microcentrifuge tube.
   - Determine gel weight by reweighing the tube containing the gel slice and subtracting the weight of the empty tube.

5. To the microcentrifuge tube, add 300 µl Binding Buffer for every 100 mg agarose gel in the tube.

6. Dissolve agarose gel slice in order to release the DNA.
   - Vortex the microcentrifuge tube 15 - 30 s to resuspend the gel slice in the Binding Buffer.
   - Incubate the suspension for 10 min at 56°C.
   - Vortex the tube briefly every 2 - 3 min during incubation.

❼ After the agarose gel slice is completely dissolved:
- Add 100 µl Binding Enhancer for every 100 mg agarose gel slice in the tube.
- Vortex thoroughly.
- Centrifuge the mixture (dissolved agarose gel slice in Binding Buffer + Binding Enhancer) briefly.

❽
- Insert one High Pure Filter Tube into one Collection Tube.
- Using a pipette, transfer the sample from step 7 to the upper reservoir of the Filter Tube.
- Centrifuge 30–60 s at 8000 × *g* in a standard table top centrifuge at +15 to +25°C.

❾
- Disconnect the Filter Tube, and discard the flowthrough solution.
- Reconnect the Filter Tube to the same Collection Tube.

❿
- Add 400 µl Wash Buffer to the upper reservoir.
- Centrifuge 30–60 s at 8000 × *g* (as above).

⓫
- Discard the flowthrough solution.
- Reconnect the Filter Tube to the same Collection Tube.
- Add 300 µl Wash Buffer.
- Centrifuge 30–60 s at 8000 × *g* (as above).

⚠ This second (300 µl) wash step ensures optimal purity.

⓬
- Discard the flowthrough solution.
- Reconnect the Filter Tube to the same Collection Tube.
- Centrifuge 1 min at maximum speed.
- Discard the flowthrough solution and Collection Tube.
- Connect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

⓭
- Add 10–20 µl Elution Buffer to the centre of the Filter Tube.
- Centrifuge 1 min at 8000 × *g*.

⚠ Do not use water for elution since alkaline pH is required for optimal yield.

⓮ The microcentrifuge tube now contains the purified DNA.

⚠ If you plan to determine the A_{260} of the eluted DNA, first centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers, which may interfere with the absorbance measurement. Use an aliquot of the supernatant to determine concentration

Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or -15 to -25°C for later analysis.

* *available from Roche Applied Science*

3. Results

Purity

Purified DNA is free of contaminants [including primers, salts, unincorporated nucleotides and proteins (*e.g.,* thermostable enzymes)]. Depending on the amount of Binding Enhancer used, the procedure may also remove small DNA fragments (< 100 bp) from the sample.

Expected Yield

Recovery of purified DNA depends on the amount of DNA loaded, elution volume, and fragment size. The table below shows the expected yields for various amounts of sample DNA.
The sample DNA was DNA Molecular Weight Marker VI* dissolved in 100 µl 10 mM Tris-HCl, pH 8.5. The experiment was performed with 200 µl Binding Buffer and 200 µl Binding Enhancer. Elution was performed with 20 µl Elution Buffer.

Tab. 1: Recovery of molecular weight marker VI.

DNA MWM VI applied (µg)	Recovery (%)
1	96
2	96
10	91
15	90
20	95

3.1 Purification of a DNA Fragment Mixture Using Various Amounts of Binding Enhancer

To evaluate the ability of the procedure to eliminate small fragments, 1.5 µg of a DNA mixture that contained a 50 bp fragment and a 400 bp fragment was purified in the presence of different amounts of Binding Enhancer (procedure 2.3). To determine the DNA yield, the absorbance of the purified samples was measured. The amount of each DNA fragment in the product mixture was determined by calculating the peak areas obtained with an Agilent Bioanalyzer® on a DNA 1000 chip. The relative proportion of both fragments in the eluate is shown in table 2.

Tab. 2: Recovery of DNA mixture containing 50 bp and 400 bp fragments.

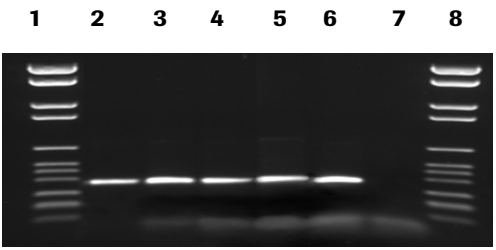
Procedure used	Amount of 50 bp fraction (%)	Amount of 400 bp fraction (%)
0% Binding Enhancer	3	97
20% Binding Enhancer	52	48
40% Binding Enhancer	54	46
Competitor Q	51	49
Competitor MN	47	53
Competitor P	44	56

As shown in table 2, the High Pure PCR Cleanup Micro Kit removes small DNA fragments very efficiently, especially in the absence of Binding Enhancer. This property of the kit can be especially useful when DNA fragments are to be used in downstream applications like rePCR, cloning or sequencing.

3.2 Purification of PCR Fragments Using Various Amounts of Binding Enhancer

A 341 bp PCR fragment of the tPA gene was amplified according to a standard block cycler protocol. The resulting reaction mixes were pooled and purified with the kit. Different amounts of Binding Enhancer were used in the purification procedure.
Portions of the PCR product (250 ng each, lane 2 – 5) and the PCR product mix (16 µl each, lane 6 – 7) were analyzed on a 1% agarose gel (Figure 1). The yield from each purification is shown in Table 3.

Fig. 1: 1% agarose gel electrophoresis of 341 bp PCR product recovered in the presence of different amounts of Binding Enhancer.



Lane 1: Molecular weight marker VI
Lane 2: 0% Binding Enhancer
Lane 3: 10% Binding Enhancer
Lane 4: 20% Binding Enhancer
Lane 5: 40% Binding Enhancer
Lane 6: PCR without purification
Lane 7: PCR negative control (PCR without template)
Lane 8: Molecular weight marker VI

Tab. 3: Yield of DNA fragments isolated in the presence of increasing amounts of Binding Enhancer.

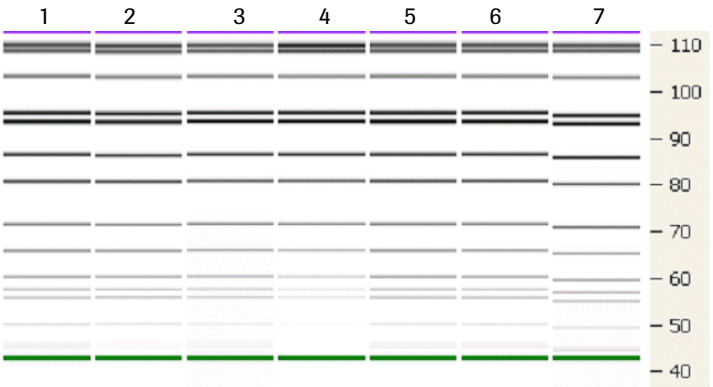
Binding Enhancer (%)	Ratio $_{260/280}$	Yield (µg)
0	1.9	0.6
10	1.9	0.9
20	1.8	1.2
40	1.9	1.7

As seen in table 3, increasing the amount of Binding Enhancer in the isolation procedure increases the recovery of DNA fragments from the PCR product mix. As shown in lanes 2-5 of figure 1, however, this increase mainly reflects the differing amounts of smaller PCR fragments (*e.g.*, primer-dimers) co-purified with the PCR product.

3.3 Purification of a Molecular Weight Marker

Molecular weight marker VIII* [containing DNA fragments of various sizes (from 19 to 1114 bp)], was dissolved in 1× Taq Polymerase buffer including $MgCl_2$* and purified with the High Pure PCR Cleanup Micro Kit in the presence of different amounts of Binding Enhancer. More than 90% of the molecular weight marker was recovered in all test samples except the one prepared without Binding Enhancer, where the recovery was only 40%. Fragment distribution in the samples is shown in figure 2.

Fig. 2 : Electropherogram of the DNA fragment distribution obtained from a Agilent Bioanalyzer run (Agilent, USA).



Lane 1: Competitor Q
Lane 2: Competitor MN
Lane 3: Competitor P
Lane 4: Roche; 0% Binding Enhancer
Lane 5: Roche; 20 % Binding Enhancer
Lane 6: Roche: 40% Binding Enhancer
Lane 7: Molecular weight marker VIII, before purification

Figure 2 shows the size exclusion possibilities with the High Pure PCR Cleanup Micro Kit.

Most procedures, including the High Pure PCR Cleanup Micro Kit procedure performed with 20% or 40% Binding Enhancer, purify DNA fragments smaller than 147 bp. However, the High Pure PCR Cleanup Micro Kit procedure without Binding Enhancer efficiently removes these small DNA fragments.

3.4 Purification of cDNA from RT Reactions

One µg of neo mRNA was reverse transcribed into cDNA with the Microarray cDNA Labeling Kit*. After cDNA synthesis was complete, 2.2 nmol of Cy5 dUTP was added to the reaction and the mixture was purified in the presence of different amounts of Binding Enhancer. After each purification, the absorbance of the eluate was measured with a NanoDrop® instrument. Different wavelengths were used to detect the nucleic acid and the Cy5 label.

Tab. 4: Yield of cDNA and adsorbance of remaining Cy5 dUTP.

Sample ID	Yield (µg)	Ratio $_{260/280}$	Cy5 value (pmol)
Competitor Q	0.1	1.9	not detectable
Competitor P	1.0	1.9	70
Competitor MN	1.1	2.0	7
Roche: 20% Binding Enhancer	1.8	2.1	not detectable
Roche: 40% Binding Enhancer	1.9	2.1	not detectable

When the High Pure PCR Cleanup Micro Kit is used to purify a cDNA synthesis/labeling reaction, the recovery of cDNA is very good and unincorporated nucleotides are completely removed.

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4. Troubleshooting

	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.
	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.
	Ethanol not added to Wash Buffer.	• Add absolute ethanol to the Wash Buffer before using. • After adding ethanol, mix the Wash Buffer well and store at +15 to +25°C. • Always label the Wash 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.
	Insufficient Binding Enhancer was used for the volume of the DNA sample.	• Add more Binding Enhancer. The maximum ratio of PCR sample : Binding Buffer : Binding Enhancer is 1:2:2. Gel loading dye does not interfere with the purification procedure.
Purified PCR product not readily cleaved by restriction enzymes.	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 centrifuge this sample tube for 1 minute at maximum speed. 2. Transfer supernatant into a new tube with out disturbing the glass fibers at the bottom of the original tube.
Absorbance (A_{260}) reading of product too high	Glass fibers, which can coelute with nucleic acid, scatter light..	See suggestions under “Purified PCR product not readily cleaved by restriction enzymes” above.
Purified DNA sample cannot easily be loaded into the well of an agarose gel, but instead „pops out“ ot 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 touch the bottom of the High Pure Filter Tube. 2. If this has occurred, empty the Collection Tube and reinsert the contaminated filter, then recentrifuge for 30 seconds.

	Possible Cause	Recommendation
Low recovery of nucleic acids in eluate	Non-optimal reagent has been used for elution	• Do not use water to elute nucleic acids from Filter Tube. Alkaline pH is required for optimal elution. • Use the Elution Buffer in the kit.
	Incomplete elution	Elute DNA with two volumes of Elution Buffer (▷10 µl each). Be sure to centrifuge after each addition of Elution Buffer.
Concentration of DNA in the eluate is too low	Low concentrations of amplified DNA were added to the High Pure Filter Tube (in Step 1)	Verify PCR result by agarose gel electrophoresis before starting purification procedure.
		Do not use less than 10 µl Elution Buffer.
No PCR product in final eluate	No PCR product in starting material	Verify PCR result by agarose gel electrophoresis before starting purification procedure.
Short read length in sequencing reactions	Copurification of DNA binding proteins	Use the Expand High Fidelity PCR System* for template synthesis.
	Copurification of primer and primer-dimer	Use Binding buffer without Binding Enhancer for purification.

** available from Roche Applied Science*

5. Additional Information on this Product

How this Product Works

In the presence of the chaotropic salt guanidine thiocyanate, DNA amplified by PCR binds selectively to special glass fibers pre-packed in the High Pure Micro Filter Tube. Bound DNA is purified in a series of rapid wash-and-spin steps to remove contaminating primers, nucleotides, and salts, and then eluted with a low salt solution. This simple method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

Test Principle

①	Sample (*e.g.*, PCR reaction is mixed with Binding Buffer and optional Binding Enhancer and purified according to kit manual. (For selection please refer to section 2.2 Application Selection Guide).
②	PCR reaction product is bound to the glass fibers pre-packed in the High Pure Filter Tube.
③	Bound DNA is washed to remove unincorporated nucleotides, primers, salts, and the thermostable polymerase.
④	Purified DNA is recovered using the Elution Buffer.

Product Characteristics

Specificity: The special glass fiber fleece binds nucleic acids from 20 bases up to > 5 kb according to the amount of binding enhancer used.

Recovery: The amount of nucleic acid recovered depends on the amount of nucleic acid applied to the glass fiber fleece, the elution volume, and the length of the PCR products. When 0.5 - 20 µg DNA is applied to the High Pure Filter Tube, >80% of the DNA can be recovered.

Quality Control

More than 70% of DNA is recovered when 3 µg DNA Molecular Weight Marker VI* is applied to the High Pure Micro Filter Tubes. Gel electrophoresis of the eluate after a purification of a 341 bp tPA gene PCR product confirms the complete removal of primer dimers. Eluted, purified DNA can readily be amplified in a LightCycler® Instrument with the LightCycler® Fast Start DNA Master^{PLUS} SYBR Green I; amplification is not inhibited.

* *available from Roche Applied Science*

References

1 Löbner, K. et al. (2002) Different Regulated Expression of the Tyrosine Phosphatase-Like Proteins IA-2 and Phogrin by Glucose and Insulin in Pancreatic Islets *Diabetes* **51**, 2982-2988.

2 Chang, PC et al. (2001) Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks *J. Gen. Virol.* **82,** 2157-2168.

3 Footitt, S. et al. (2003) Expression of the viviparous 1 (Pavp1) and p34cdc2 protein kinase (cdc2Pa) genes during somatic embryogenesis in Norway spruce (Picea abies [L.] Karst) *J. Exp. Bot.* **54** (388), 1711 - 1719.

4 Gilad, Y. et al. (2003) Human specific loss of olfactory receptor genes *Proc Natl Acad Sci USA* **100** (6): 3324-3327.

Further Readings

5 Vogelstein, B. et al. (1979) Preparative and analytical purification of DNA from agarose *Proc Natl Acad Sci USA* **76** (2):615-619.

6 Aitken, N. et al. (2004) Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach *Molecular Ecology* **13** (6): 1423-1431.

7 Behnke, A. et al. (2004) Reproductive Compatibility and RDNA Sequence Analysis in the Sellaphora Pupula Species Complex (Bacillariophyta) *Journal of Phycology* **40** (1), 193-208.

8 Diegisser, T. et al. (2004) Genetic and morphological differentiation in Tephritisbardanae (Diptera: Tephritidae): evidence for host-race formation *Journal of Evolutionary Biology* **17 (**1): 83-89.

9 D'Errico, I. et al. (2005) Study of the mitochondrial transcription factor A (Tfam) gene in the primate Presbytis cristata *Gene* **354:** 83-89.

6. Supplementary Information

6.1 Conventions

6.1.1 Text Conventions

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

Text Convention	Usage
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.

6.1.2 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.

6.1.3 Changes to previous version

Editorial changes

6.2 Ordering Information

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:

- DNA & RNA preparation - Versatile Tools for Nucleic Acid Purification: http:// www.roche-applied-science.com/napure
- PCR - Innovative Tools for Amplification: http:// www.roche-applied-science.com/pcr

	Product	Pack Size	Cat. No.
Associated Kits	PCR Cloning Kit (blunt end)	35 reactions and 5 control reactions	11 939 645 001
	Rapid DNA Ligation Kit	40 DNA ligation reactions	11 635 379 001
	SP6/T7 Transcription Kit	2× 20 transcription reactions	10 999 644 001
	Microarray cDNA Labeling Kit	25 reactions	04 401 557 001
Single reagents	Agarose MP (multi purpose agarose)	100 g 500 g	11 388 983 001 11 388 991 001
	Agarose LE (low electroendosmosis)	100 g 500 g	11 816 586 001 11 816 594 001
	Agarose MS (molecular screening agarose)	100 g 500 g	11 685 660 001 11 685 678 001
	T4 DNA Ligase	100 U	10 481 220 001
	rAPid Alkaline Phosphatase	1000 U 5000 U	04 898 133 001 04 898 141 001
	Expand High Fidelity PCR System	100 U 500 U 2,500 U	11 732 641 001 11 732 650 001 11 759 078 001

6.3 Trademarks

Regulatory Disclaimer

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- Pack Inserts and Product Instructions

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