

**Nucleic Acid Isolation and Purification
Product Selection Guide**
On the Hunt for Success



Perform great nucleic acid isolation and purification

Start your experiments right off by generating high-purity nucleic acids with Roche Applied Science's nucleic acid isolation and purification kits. Follow the product selection guide on pages 4 and 5 of this brochure to choose the best kit. Then perform robust, reproducible purification of DNA and RNA for use in genomic and classical molecular biology techniques.

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Roche Applied Science has a wide array of manual nucleic acid preparation products that are specifically designed to prepare DNA or RNA templates from a particular source or for certain applications (see “Overview” on pages 6 – 9).

Our nucleic acid purification products combine proven, reliable purification methods with innovations that allow you to:

- Process more samples in less time
- Minimize nucleic acid loss and degradation
- Increase laboratory efficiency and safety
- Avoid organic solvents and toxic reagents
- Isolate many types of nucleic acids from different sample materials

- Achieve highly purified nucleic acids of high integrity, suitable for amplification
- Obtain reliable, reproducible performance

Roche Applied Science uses ISO-certified operating and testing procedures for the HIGH PURE product line, thus ensuring that the performance of each lot of each product is similar to the next lot.

Product Selection Guide

Use this table to select a product according to the type of nucleic acid you wish to purify, then consider the source of the nucleic acid and the scale of the purification.

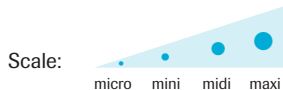
Purification of DNA

Nucleic Acid Type	Subtype	Origin/Source	Scale	Recommended Product	Cat. No.			
DNA	Genomic	tissue, cultured cells, bacteria, yeast, blood	•	High Pure PCR Template Preparation Kit	11 796 828 001			
		tissue, cultured cells, bacteria, yeast, mouse tail	• •	DNA Isolation Kit for Cells and Tissues	11 814 770 001			
		human blood	•	High Pure PCR Template Preparation Kit	11 796 828 001			
		mammalian/human blood	• •	DNA Isolation Kit for Mammalian Blood	11 667 327 001			
	Plasmid	propagated in <i>E. coli</i>	•	High Pure Plasmid Isolation Kit	(50 purifications) 11 754 777 001 (250 purifications) 11 754 785 001			
		propagated in <i>E. coli</i>	•	Genopure Plasmid Midi Kit	03 143 414 001			
		propagated in <i>E. coli</i>	•	Genopure Plasmid Maxi Kit	03 143 422 001			
	Viral	serum, plasma, blood, other body fluids, supernatant from cell cultures	•	High Pure Viral Nucleic Acid Kit	11 858 874 001			
			•	High Pure Viral Nucleic Acid Large Volume Kit	05 114 403 001			
		serum, plasma, supernatant from cell cultures	•	High Pure 16 System Viral Nucleic Acid Kit	12 011 816 001			
	DNA fragments	PCR mixture	•	High Pure PCR Product Purification Kit	(50 purifications) 11 732 668 001 (250 purifications) 11 732 676 001			
				High Pure PCR Cleanup Micro Kit	(50 purifications) 04 983 955 001 (200 purifications) 04 983 912 001			
			•	High Pure 96 UF Cleanup Kit	04 422 694 001			
			restriction enzyme digests, labeling and modifying reaction mixture	•	High Pure PCR Product Purification Kit	(50 purifications) 11 732 668 001 (250 purifications) 11 732 676 001		
		•		High Pure PCR Cleanup Micro Kit	(50 purifications) 04 983 955 001 (200 purifications) 04 983 912 001			
		radiolabeled DNA	•	Quick Spin Columns	(20 columns, G-25) 11 273 922 001 (50 columns, G-25) 11 273 949 001 (20 columns, G-50) 11 273 965 001 (50 columns, G-50) 11 273 973 001			
					mini Quick Spin Columns	11 814 419 001		
					agarose gel slices	• •	Agarose Gel DNA Extraction Kit	11 696 505 001
						•	High Pure PCR Product Purification Kit	(50 purifications) 11 732 668 001 (250 purifications) 11 732 676 001
		ss DNA, ds DNA, PCR products, cRNA	•	Microarray Target Purification Kit	03 266 885 001			
				High Pure PCR Cleanup Micro Kit	(50 purifications) 04 983 955 001 (250 purifications) 04 983 912 001			

Product Selection Guide, continued

Purification of RNA

Nucleic Acid Type	Subtype	Origin/Source	Scale	Recommended Product	Cat. No.
RNA	mRNA	cultured cells, tissues, total RNA	• •	mRNA Isolation Kit	11 741 985 001
			• •	mRNA Capture Kit	11 787 896 001
		whole blood/bone marrow	• • •	mRNA Isolation Kit for Blood/Bone Marrow	11 934 333 001
	Total RNA	cultured cells, bacteria, yeast, blood	•	High Pure RNA Isolation Kit	11 828 665 001
			•	High Pure RNA Tissue Kit	12 033 674 001
		cultured cells, tissues, bacteria, yeast, blood, plant cells	• • •	TriPure Isolation Reagent	(50 ml) 11 667 157 001 (200 ml) 11 667 165 001
			•	High Pure FFPE RNA Micro Kit	04 823 125 001
		formalin-fixed paraffin-embedded tissue, fresh-frozen tissue	•	High Pure RNA Paraffin Kit	03 270 289 001
		animal tissue, stabilized animal tissue, plant tissue, animal cell culture, formalin-fixed, paraffin-embedded tissue	•	High Pure miRNA Isolation Kit	05 080 576 001
		Viral RNA	serum, plasma, other body fluids, supernatant from cell cultures	•	High Pure Viral RNA Kit
	radiolabeled RNA			•	Quick Spin Columns
		RNA fragments		•	mini Quick Spin Columns



Product Overview

Detailed Product Characteristics

	Purification Method/Product	Starting Material and Quantity	Yield/Recovery	Time Required	PCR/Long PCR	RT-PCR	Differential Display RT-PCR
Ultrafiltration	High Pure 96 UF Cleanup Kit	PCR products (100 bp to > 10 kb), 20-300 µl	≥25 µl (≥150 bp, ≥40%; 1500 bp, ≥90%; 4500 bp, ≥90%, 8000 bp, ≥80%)	< 20 min			
	High Pure PCR Template Preparation Kit	blood, 200-300 µl	3-9 µg	20 min	■		
		cultured cells, 10 ⁴ - 10 ⁸	15-20 µg	20 min			
		thymus tissue, 25-50 mg mouse tail, 25-50 mg	5-20 µg 5-10 µg	80 min, incl. lysis 200 min, incl. lysis			
	yeast, 10 ⁸ cells	10-13 µg	50 min, incl. lyticase digest				
	bacteria, 10 ⁹ cells	1-3 µg	35 min, incl. lysozyme digest				
	High Pure PCR Product Purification Kit	PCR, modifying, labeling, restriction digestion reaction, agarose gel slice	>80% recovery of 5-25 µg DNA, >100 bp	10 min	■		
Silica Adsorption	High Pure PCR Cleanup Micro Kit	PCR, modifying, labeling, restriction digestion reaction, agarose gel slices	>85% recovery up to 20 µg DNA	10 min	■		
	High Pure Plasmid Isolation Kit	<i>E. coli</i> XL1 blue, pUC19 (2 ml)	12 µg	30 min	■		
		<i>E. coli</i> HB 101, pUC19 (2 ml)	6 µg	30 min			
		<i>E. coli</i> DH5α, pUC19 (2 ml)	3.5 µg	30 min			
	High Pure RNA Isolation Kit	blood, 200-500 µl	sufficient for 10 RT-PCR reactions	50 min, incl. RBC lysis		■	■
		cultured cells, 10 ⁶	20 µg	25 min			
		yeast, 10 ⁸ cells	20 µg	45 min, incl. lyticase digest			
		bacteria, 10 ⁹ cells	35-50 µg	90 min, incl. lysozyme digest			
	High Pure RNA Tissue Kit	tissue, 1-10 mg	0.5-3.0 µg/mg	30 min		■	■
	High Pure miRNA Isolation Kit	tissue, stabilized tissue, plant tissue, cell culture, FFPE tissue sections	depending on miRNA	30 min		■	
High Pure Viral RNA Kit	serum, plasma, urine, supernatant from cell culture, 200-600 µl	product detectable by RT-PCR	10 min		■		
High Pure Viral Nucleic Acid Kit	serum, plasma, blood, supernatant from cell culture, 200-600 µl	product detectable by PCR or RT-PCR	20 min	■	■		
High Pure 16 System Viral Nucleic Acid Kit	serum, plasma, supernatant from cell culture, 200 µl	product detectable by PCR or RT-PCR	30 min	■	■		
High Pure Viral Nucleic Acid Large Volume Kit	serum, plasma, whole blood	25 ml	25 min	■	■		
Ion-Exchange Chromatography	Microarray Target Purification Kit	cDNA synthesis, amplification, <i>in vitro</i> transcription reactions	ds DNA 95%, cRNA >80%	30 min			
	High Pure RNA Paraffin Kit	fresh-frozen or formalin-fixed paraffin-embedded tissue	0.3-1.5 µg/5 µm section, 2-6 µg/20 mg fresh-frozen tissue	2 h without overnight incubation		■	
	High Pure FFPE RNA Micro Kit	1-10 µm FFPE tissue sections	1.5-3.5 µg/5 µm	60 min without 3h incubation		■	■
	Agarose Gel DNA Extraction Kit	agarose gel slices, 100-200 mg	80% recovery of fragments (0.4-9.5 kb)	60 min	■		
	Genopure Plasmid Midi Kit	<i>E. coli</i> DH5α, pBS (30 ml)	>85 µg	60 min	■		
	Genopure Plasmid Maxi Kit	<i>E. coli</i> DH5α, pBS (150 ml)	>420 µg	75 min	■		

Product Overview

Recommended Uses

cDNA Synthesis/ Primer Extension	RE Digestion	Southern Blotting	Labeling, Modifying Reactions	Northern Blotting	RNase Protection Assays	Cloning	Sequencing	<i>In vitro</i> Transcription	<i>In vitro</i> Translation	Transfection	Microarray Spotting
	■		■			■	■				■
	■	■				■					
	■	■	■			■	■	■			
	■	■	■			■	■	■			
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	■	■				■	■			■	

Product
Overview

Product Overview, continued

Detailed Product Characteristics

Purification Method/Product	Starting Material and Quantity	Yield/Recovery	Time Required	PCR/Long PCR	RT-PCR	Differential Display RT-PCR	
Affinity Purification	mRNA Isolation Kit for Blood/Bone Marrow	blood, bone marrow aspirate, 1.5-5 ml	50-200 ng/ml blood	90 min		■	
	mRNA Isolation Kit	tissue, 50 mg-1 g	7-14 µg/100 mg tissue	60 min		■	
		cells, 2×10^5 - 10^8 total RNA, 250 µg-2.5 mg	0.3-25 µg/ 10^7 cells 1-5 µg/100 µg total RNA	40 min 30 min			
mRNA Capture Kit	tissue, up to 20 mg cells, up to 5×10^5 total RNA, up to 40 µg	product detectable by RT-PCR product detectable by RT-PCR product detectable by RT-PCR	60 min 40 min 30 min		■		
Solution-based Isolation	DNA Isolation Kit for Cells and Tissues	tissue, 100 mg-1 g cultured cells, 1×10^7 - 5×10^7 mouse tail, 50-400 mg yeast, up to 3×10^{10} bacteria, up to 1×10^{11}	depending on tissue type 700-3000 µg/ 5×10^7 cells 800 µg/400 mg mouse tail 300 µg/ 3×10^{10} yeast cells 1500-2750 µg/ 10^{11} bacteria	2.5 h, excl. resuspension	■		
	DNA Isolation Kit for Mammalian Blood	human whole blood, 10 ml	350 µg	1.5 h, excl. resuspension	■		
		mouse/rat whole blood, 10 ml	570 µg	1.5 h, excl. resuspension			
	TriPure Isolation Reagent	RNA from liver, spleen, 50 mg-1 g	6-10 µg/mg tissue	2.5 h		■	■
		RNA from cultured epithelial cells, 10^6 - 10^7	8-15 µg/ 10^6 cells	2.5 h			
	DNA from liver, kidney, brain, 50 mg-1 g DNA from cultured cells, human, rat, 10^6 - 10^7	2-4 µg/mg tissue 5-7 µg/ 10^6 cells	3.5 h 3.5 h	■			
Gel Filtration	Quick Spin Columns for radiolabeled DNA purification, Sephadex G-25	up to 50 µl labeling mixture	>80%	10 min			
	Quick Spin Columns for radiolabeled DNA purification, Sephadex G-50	up to 100 µl labeling mixture	>90%	10 min			
	Quick Spin Columns for radiolabeled RNA purification, Sephadex G-25	up to 50 µl labeling mixture	>80%	10 min			
	Quick Spin Columns for radiolabeled RNA purification, Sephadex G-50	up to 100 µl labeling mixture	>80%	10 min			
	mini Quick Spin DNA Columns	20 - 75 µl labeling mixture	>90%	7 min			
	mini Quick Spin RNA Columns	20 - 75 µl labeling mixture	>80%	7 min			
	mini Quick Spin Oligo Columns	20 - 50 µl labeling mixture	>80%	7 min			

Product Overview

Recommended Uses

cDNA Synthesis/ Primer Extension	RE Digestion	Southern Blotting	Labeling, Modifying Reactions	Northern Blotting	RNase Protection Assays	Cloning	Sequencing	<i>In vitro</i> Transcription	<i>In vitro</i> Translation	Transfection	Microarray Spotting
■				■	■				■		
■				■	■				■		
	■	■				■					
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Product
Overview

Smart RNA Isolation

Flexibility unlimited

High Pure FFPE RNA Micro Kit

Choose the High Pure FFPE RNA Micro Kit to isolate total RNA from 1- to 10- μ m sections of formalin-fixed, paraffin-embedded (FFPE) tissue samples (e.g., colon, breast, liver, kidney, and spleen of mammalian species including human research samples) for direct use in RT-PCR. Isolate and purify small sample amounts using a fast and convenient workflow. The isolated RNA is suitable for relative quantification of mRNA in RT-PCR using the LightCycler® Instruments or other real-time PCR systems (Figure 2).

- **Rely on effective functioning.**

Use the simple and rapid kit protocol for consistent recovery of RNA suitable for qRT-PCR.

- **Achieve high purity.**

Novel optimized columns produce highly concentrated (10 μ l) eluate and high recovery ($\geq 80\%$) rates of total RNA – even small RNA fragments.

- **Insist on high sensitivity.**

Generate high-quality template RNA that shows excellent performance and linearity in RT-PCR.

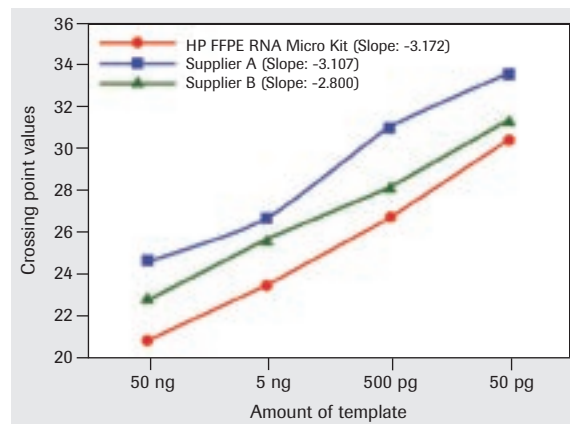
Product	Cat. No	Pack Size
High Pure FFPE RNA Micro Kit	04 823 125 001	Up to 50 isolations

Figure 2: CP values. LightCycler® 1.5 Instrument crossing point (CP) values of a $\beta 2$ -microtubulin specific RT-PCR in relation to sample amount.

Benefit from a smart device



Figure 1: Cross-section view. A reducing device, designed as collecting funnel, creates a cavity for the silica membrane at the bottom of the spin column with a binding capacity of 20 μ g. The special design of the blue colored reducing device allows easy central loading of sample and buffer up to 500 μ l. Due to the funnel-like design, carry-over contamination is avoided. A foot-hold membrane prevents the silica membrane slipping through the outlet.



High Pure miRNA Isolation Kit

MicroRNAs are natural regulators of gene expression, affecting almost every cellular process (growth and development, cell differentiation, cell death). They occur naturally in diverse animal and plant species, and hundreds have been discovered since the late 1990s. A single miRNA can ramp down expression of multiple genes.

The kit is designed for the isolation and enrichment of small RNA (*e.g.*, miRNA / microRNA) from animal cells, tissue samples or formalin-fixed, paraffin-embedded tissue. It can be used to purify total RNA or to prepare samples enriched for small RNA <100 nucleotides. The quality of the preparation is suitable for Northern Blotting, miRNA Array hybridization and for the relative quantification of miRNA with RT-PCR (*e.g.*, on the LightCycler® 480 System).

- **Isolate DNA-free miRNA.**
Ideal for qualitative and quantitative reverse transcription.
- **Obtain excellent performance.**
By taking an optimized protocol for high yield miRNA.
- **Save time.**
With a simple, organic-free and efficient protocol.
- **Generate stable, highly pure miRNAs.**

Product	Cat. No	Pack Size
High Pure miRNA Isolation Kit	05 080 576 001	Up to 50 miRNA isolations

High Pure RNA Isolation Kit

Choose the High Pure RNA Isolation Kit for rapid isolation of intact total RNA from mammalian cultured cells, mammalian blood, white blood cells, yeast, gram positive bacteria and gram negative bacteria.

The RNA obtained is ideal for many subsequent molecular biology techniques, especially those that require the absence of genomic DNA. The intact, total RNA is ready-to-use in techniques like cDNA library construction, RT-PCR, northern blotting, differential display, nuclease protection assay, primer extension, RACE, and *in vitro* translation (Figure 2).

- **Process a wide variety of sample material.**
Use this versatile kit for all your RNA isolations.
- **Obtain high RNA yields in less time.**
Produce high yields of RNA from multiple samples in minutes.
- **Avoid genomic DNA contamination.**
Integrated DNA digestion and DNase removal eliminate signals from genomic DNA.
- **Purify RNA for applications other than RT-PCR.**
Prepare total RNA for use in microarray target generation, northern blotting, primer extension assays, RACE, *in vitro* translation, or nuclease protection assays.

Product	Cat. No.	Pack Size
High Pure RNA Isolation Kit	11 828 665 001	Up to 50 reactions

Smart RNA Isolation

Flexibility unlimited

mRNA Capture Kit

Choose the mRNA Capture Kit for capturing of polyadenylated RNA from total RNA, cell lysates and tissue homogenates in 200 µl PCR tubes without prior RNA preparation. The method relies on the base-pairing between the Poly (A+) residues at the 3'p-end of the mRNA and kit's biotin-labeled oligo (dT) probe. The formed hybrid is then immobilized in the streptavidin-coated PCR tubes, and unbound contaminants removed by wash steps. The immobilized biotin-labeled oligo (dT)/poly (A+) hybrid can serve as a primer for the reverse transcriptase step in RT-PCR in the same tube (Figure 4).

- Maximize convenience.**
 Combine rapid, efficient isolation of poly (A+) RNA with RT-PCR in a single tube to prevent sample loss, reduce handling time, and minimize the risk of contamination.
- Save time.**
 Isolate RNA with an innovative process that simplifies the handling of a large number of samples.
- Improve reliability and reproducibility.**
 Produce excellent RT-PCR templates even from small amounts of starting material, or material containing low-abundance mRNAs.

Product	Cat. No.	Pack Size
mRNA Capture Kit	11 787 896 001	192 isolations

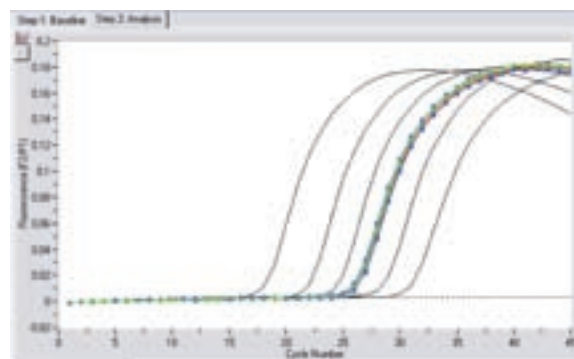


Figure 3: Sensitive low copy RNA quantification. White blood cells from three different human research samples were prepared from EDTA-blood using Red Blood Cell Lysis Buffer. Cells were counted and adjusted. RNA from about 3×10^6 white blood cells of each donor (triplicates; colored lines with dots, squares, crosses) were isolated using the High Pure RNA Isolation Kit and analyzed by the LightCycler® h-HPRT Housekeeping Gene Set. The pre-diluted standard solutions of *in vitro* transcribed RNA of Hypoxanthine-phosphoribosyl-transferase (HPRT) was used for the reference curve (grey lines).

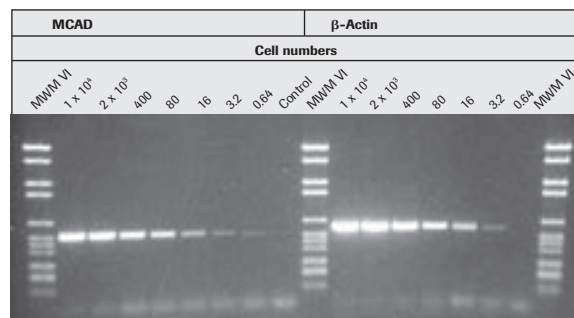


Figure 4: Detection of MCAD and β -actin transcripts in K562 cells. Lysates from 10^6 cells were serially diluted. Messenger RNA from the dilutions was captured in Streptavidin-coated PCR tubes according to the kit protocol. RT-PCR was performed in the same PCR tubes with reagents from the Titan One Tube PCR System and primers derived from the human MCAD (Medium-chain acyl-CoA dehydrogenase) and β -actin genes. Amplicons were run on a 1% agarose gel.

Optimized DNA Isolation and Purification

Flexibility unlimited

High Pure PCR Template Preparation Kit

Choose the versatile High Pure PCR Template Preparation Kit to isolate genomic DNA from whole blood, buffy coat, cultured cells, tissue, yeast, bacteria, mouse tail, and other sample materials. Multiple PCR templates can be obtained in minutes with this fast and efficient kit. The single kit facilitates reproducibility and reliability for a multitude of applications, including standard or long template PCR, SNP detection, Southern blotting, qPCR, microarray analysis, and cloning (Figure 5).

- **Insist on unlimited flexibility.**
A single versatile kit purifies nucleic acids from a wide variety of sample materials.
- **Enhance amplification results.**
PCR inhibitors, which can interfere with the amplification reaction, are efficiently removed.
- **Facilitate long template applications.**
High molecular weight DNA (30-50 kb) can be isolated.
- **Be confident in your PCR results.**
High-quality DNA obtained using this kit delivers reproducible results, even in real-time PCR.

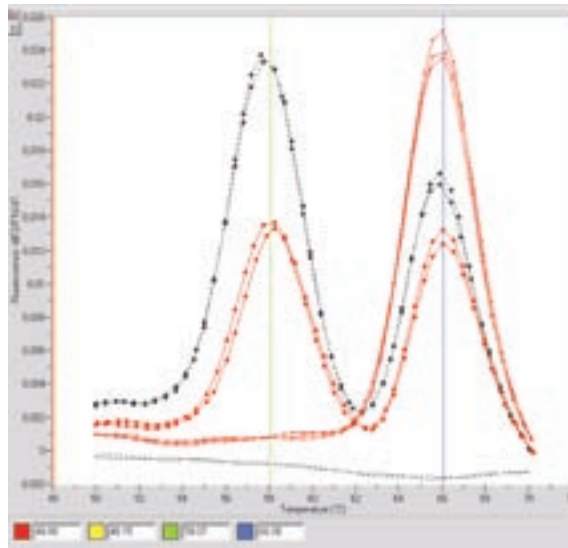


Figure 5: Melting curve analysis of the three possible genotypes of the Factor V Leiden sequence. Genomic DNA from human blood research samples were purified by the High Pure PCR Template Preparation Kit and analyzed by the LightCycler® Factor V Leiden Mutation Detection Kit for point mutations in the human Factor V gene. Water was used as negative control (black dashed line/baseline). Heterozygous plasmid DNA was used as positive control (black line with dots). The red line with crosses shows a blood sample from a homozygous donor (wildtype genotype). The red line with dots show a blood sample spiked with the heterozygous plasmid. The melting peaks indicate that the fully homologous sequence of the wildtype genotype has a higher melting temperature than the sequence of the mutant genotype. Heterozygous samples containing both sequences display two peaks.

Product	Cat. No.	Pack Size
High Pure PCR Template Preparation Kit	11 796 828 001	Up to 100 purifications

Optimized DNA Isolation and Purification

Flexibility unlimited

High Pure Viral Nucleic Acid Kit

Choose the High Pure Viral Nucleic Acid Kit to isolate viral DNA and RNA from serum, plasma, cell culture supernatant, or whole blood. The highly purified viral nucleic acids can be used directly as templates in qualitative and quantitative PCR and RT-PCR (Figure 6). When using whole blood, total nucleic acids are isolated, including viral nucleic acids.

- **Save time.**

Prepare multiple PCR/RT-PCR templates in 20 minutes, with only 10 minutes of hands-on time.

- **Accommodate a wide variety of samples.**

Use one kit for both DNA and RNA viral purification allowing simultaneous testing of both virus types.

- **Minimize loss of nucleic acids.**

Remove contaminants without precipitation, solvent extraction, or other handling steps that can lead to lost or degraded nucleic acids.

- **Obtain accurate results.**

Achieve high sensitivity and reproducibility in many applications due to the viral nucleic acid being concentrated in a 50 µl eluate.

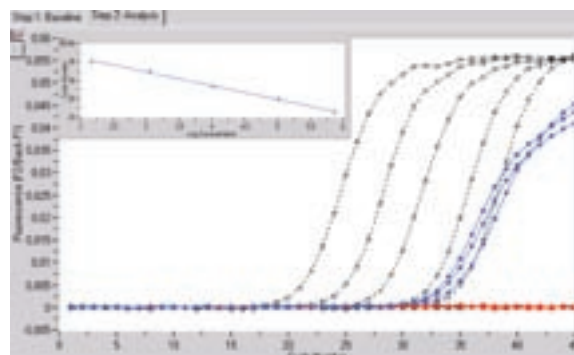


Figure 6: Quantification of Epstein-Barr virus DNA in research samples. EDTA blood samples, artificially spiked with quantified EBV virus material, were extracted using the High Pure Viral Nucleic Acid Kit and quantitatively analyzed by the LightCycler® EBV Quantification Kit. The DNA standards were ranging from 10^6 to 10^2 copies/reaction (grey line with crosses), resulting in the standard curve. The negative control (water) is indicated by a black line with squares.

EBV positive blood samples (triplicate, spiked with the EBV genome containing Namalwa cell line) correspond to the lower limit of 400 copies/reaction (blue line with dots). The negative control (blood not spiked with Namalwa cell line) is indicated by the red line with dots. The efficient nucleic acid extraction excludes misinterpretation or inhibition of the amplification reaction and is therefore well suited for the quantification of EBV virus DNA.

Product	Cat. No.	Pack Size
High Pure Viral Nucleic Acid Kit	11 858 874 001	Up to 100 purifications

High Pure Viral Nucleic Acid Large Volume Kit

Efficiently isolate total viral nucleic acids from up to 2.5 ml of mammalian whole blood, serum, and plasma with the new High Pure Viral Nucleic Acid Large Volume Kit. Obtain highly purified viral RNA or DNA using the innovative combination of a novel extender and proven High Pure technology (Figure 7). Use a simple, rapid (less than 25 minutes) protocol and optimized reagents to generate concentrated, purified nucleic acids suitable for direct use in qPCR, qRT-PCR, sequencing, cloning, and other applications.

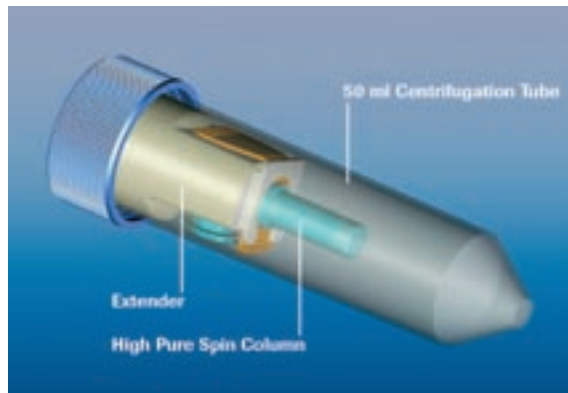


Figure 7: High Pure Extender Assembly. The ready-to-use device, pre-assembled in a 50 ml polypropylene tube, features an extender that accommodates large sample volumes. The extender is directly connected to a High Pure Mini Filter Tube and secured by a locking clip.

- **Improve sensitivity.**

Use sample volumes up to 2.5 ml to obtain high yields of purified nucleic acids in a concentrated 50 μ l eluate.

- **Obtain high-purity nucleic acids.**

Reduce carryover risk by using high centrifugal forces in all wash steps.

- **Increase convenience and improve time to result.**

Eliminate complicated sample pre-processing and rapidly recover purified nucleic acids using high-speed centrifugation.

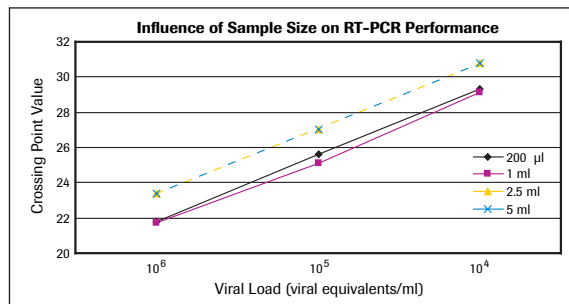


Figure 8: Purify nucleic acids from a broad range of sample sizes. Various amounts (200 μ l, 1 ml, 2.5 ml, and 5 ml) of serum research samples were spiked with a dilution series (1×10^6 to 1×10^4 copies/ml) of hepatitis A virus (HAV) particles. Nucleic acids were isolated from each sample using the High Pure Viral Nucleic Acid Large Volume Kit according to the kit protocol. Five microliters of each sample eluate was analyzed by LightCycler[®] qRT-PCR.

Result: The data shows the sensitivity and linearity of the purified nucleic acids in qRT-PCR analysis, and consistent performance independent of sample volume. The experiment also demonstrates that the High Pure Viral Nucleic Acid Large Volume Kit can accommodate serum sample volumes as large as 5 ml, yielding highly pure, concentrated nucleic acids.

Product	Cat. No.	Pack Size
High Pure Viral Nucleic Acid Large Volume Kit	05 114 403 001	Up to 40 reactions

Optimized DNA Isolation and Purification

Flexibility unlimited

DNA Isolation Kit for Mammalian Blood

Choose the DNA Isolation Kit for Mammalian Blood to easily and economically isolate genomic DNA from 1 to 10 ml mammalian whole blood. The kit provides all necessary reagents for the rapid isolation of purified genomic DNA, free of contaminating heme and proteins. The resulting DNA is pure, has a high molecular weight, and is ready for use in many applications, including genomic Southern blots and standard and long template PCR. The kit also supports isolation from buffy coat and lymphocyte samples (Figure 9).

- **Easily prepare pure DNA in less than 90 minutes.**

Purify DNA with the convenience of a kit with minimal hands-on time and no organic extraction.

- **Achieve consistent and reliable results.**

Each component of the kit is tested to be DNase free, and each kit is function-tested using the Expand Long Template PCR System.

- **Benefit from convenience and versatility.**

The kit is applicable to different sample volumes (1-10 ml), different blood anticoagulants (heparin, citrate, EDTA) and is tested for several types of mammalian whole blood (human, mouse, rat, dog, porcine, guinea pig, monkey, rabbit, bovine) and human blood components (buffy coat, lymphocytes).

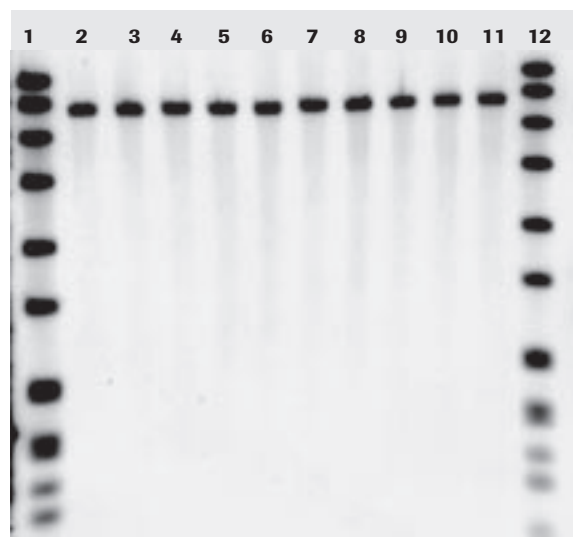


Figure 9: Detection of the *n-ras* gene by Southern hybridization. DNA preparation from several human blood research samples, each of which had been prepared with a different anticoagulant. DNA was also prepared from lymphocyte and buffy coat preparations. Ten μg of each preparation was digested with *Eco* RI, electrophoretically separated, and blotted to a nylon membrane. DNA on the membrane was hybridized to a DIG-labeled *n-ras* probe, and the results visualized chemiluminescently. Each lane contained only a single hybridization band of the expected (7.2 kb) size. The samples used were:

Lanes 1, 12: DNA Molecular Weight Marker VII

Lanes 2, 3: whole blood, sodium citrate anticoagulant

Lanes 4, 5: whole blood, heparin anticoagulant

Lanes 6, 7: whole blood, sodium EDTA anticoagulant

Lanes 8, 9: buffy coat preparation

Lanes 10, 11: lymphocyte preparation

Product	Cat No.	Pack Size
DNA Isolation Kit for Mammalian Blood	11 667 327 001	25 isolations from 10 ml blood.

Genopure Plasmid Kits

Choose from two Genopure Plasmid Kits to prepare high-quality plasmid DNA that is suitable for transfection, sequencing, restriction digest/Southern blotting, PCR, cloning and more. Isolate up to 100 µg (Genopure Plasmid Midi Kit) or up to 500 µg (Genopure Plasmid Maxi Kit) concentrated plasmid DNA for use with FuGENE® 6 Transfection Reagent or other transfection methods. Suitable for all plasmid sizes, these kits eliminate the time-consuming post-alkaline lysis centrifugation step, resulting in the removal of cellular debris and potassium–SDS precipitates in a few minutes.

Use the Genopure Buffer Set for isolation of low copy number plasmid DNA in combination with the Genopure Plasmid Kits.

- **Purify plasmid DNA with minimal hands on time.**

Process multiple samples simultaneously, using convenient high speed gravity-flow columns.

- **Perform highly reproducible purifications.**

Obtain pure plasmid DNA free of bacterial components and RNA contamination.

- **Save processing time.**

Use the ready-to-use reagents and supplied folded filters to significantly reduce preparation time.

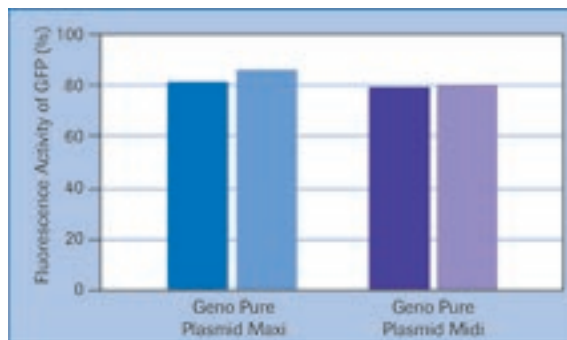


Figure 10: Quantification of GFP fluorescence activity expressed in RTS 100 HY reactions from different pIVEX2.3-GFP reactions performed in duplicate.

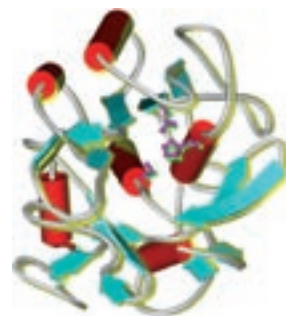
Plasmid DNA from a pIVEX construct coding for GFP and a C-terminal His₆ tag was prepared with the Genopure Plasmid Midi and Maxi Kit. The preparation results in DNA samples of high purity and excellent yields.

The plasmid kits are highly recommended for the isolation of plasmid DNA intended for use as template for all formats of the RTS as these kits meet the stringent requirements for this application.

Product	Cat. No.	Pack Size
Genopure Plasmid Midi Kit	03 143 414 001	Up to 20 preparations
Genopure Plasmid Maxi Kit	03 143 422 001	Up to 10 preparations
Genopure Buffer Set for Low-Copy Number Plasmids	04 634 772 001	1 set

Enzymes to Degrade Cellular Components

A 50-year tradition of high-quality enzymes



Proteinase K recombinant, PCR Grade

This enzyme is the recombinant form of the enzyme from *Tritirachium album* expressed in *Pichia pastoris*. The enzyme is extremely effective on native proteins and can therefore be used to rapidly inactivate endogenous nucleases during nucleic acid isolation for cloning, sequencing, PCR, and RT-PCR. This property makes Proteinase K particularly suitable for the isolation of native RNA and DNA from tissues or cell lines. The enzyme is tested for the absence of RNases and DNases and is virtually free of DNA; it is thus well suited for isolating nucleic acids for amplification reactions. The enzyme's low bioburden content guarantees improved product stability and security.

- **Choose a universal tool for template preparation.**
 Proteinase K inactivates DNases and RNases of most species.
- **Insist on stringent quality tests for contaminants and extended specification parameters.**
 Reliable and sensitive quality control tests ensure detection of trace contaminants.
- **Work with a versatile and consistent enzyme.**
 The enzyme is extremely effective on native proteins from different sources and active over a wide range of operating conditions.

Product	Cat. No.	Pack Size
Proteinase K recombinant PCR Grade (lyophilizate)	03 115 836 001	25 mg
	03 115 879 001	100 mg
	03 115 801 001	500 mg (2 x 250 mg)
	03 115 852 001	1 g (4 x 250 mg)
Proteinase K recombinant, PCR Grade (solution)	03 115 887 001	1.25 ml
	03 115 828 001	5 ml
	03 115 844 001	25 ml

DNase I recombinant, RNase-free

Easily remove even trace quantities of DNA from molecular biology reactions by using our recombinant DNase I. DNase I recombinant treatment results in high quality, intact, undegraded RNA ready for further downstream applications like RT-PCR, degradation of genomic DNA template in *in vitro* transcription reactions, labeling of DNA by nick translation, foot-printing analysis of DNA-protein interaction, and microarray target control. The highly purified enzyme is the recombinant form of bovine pancreatic DNase I produced in the host *Pichia pastoris*.

- **Eliminate DNA contamination.**
 Maintain RNA integrity and safely eliminate DNA contamination from any RNA sample.
- **Benefit from an animal-free product.**
 The entire production process and product are animal-free, eliminating concerns when handling bovine-derived material.
- **Ensure maximal activity.**
 The enzyme is supplied with an optimized incubation buffer.
- **Reduce contamination risk with reliable lot-to-lot consistency.**
 Use an enzyme preparation that is rigorously tested for RNase and protease contamination.

Product	Cat. No.	Pack Size
DNase I recombinant, RNase-free	04 716 728 001	10,000 units

Premium Post-PCR Performance

Flexibility unlimited

High Pure PCR Cleanup Micro Kit

Use the High Pure PCR Cleanup Micro Kit to efficiently purify products from PCR and other reactions. The kit eliminates contaminants from amplification reactions (e.g., primers, mineral oil, salts) and can also be used to purify nucleic acids from other modification reactions such as restriction endonuclease digests, alkaline phosphatase treatment, and kinase reactions. In addition, the kit can be utilized to purify cDNA, concentrate dilute nucleic acid solutions, and recover DNA from agarose gel slices. The purified DNA can be used directly in subsequent enzymatic reactions such as labeling, sequencing, cloning, and ligation, as well as for PCR analysis.

- **Conserve resources.**

Use one versatile kit that eliminates the need to use several kits from other suppliers.

- **Save time.**

Use a simple and rapid protocol that reduces purification time.

- **Obtain purified product in a small elution volume ($\leq 10 \mu\text{l}$).**

Use it for demanding downstream applications.

- **Efficiently remove contaminants and unwanted reaction components.**

- **Generate contaminant-free DNA.**

Use it for direct cloning, ligation, restriction digests, and other reactions.

- **Selectively isolate specific DNA fragment sizes.**

Use the kit's binding enhancer to adjust purification stringency.

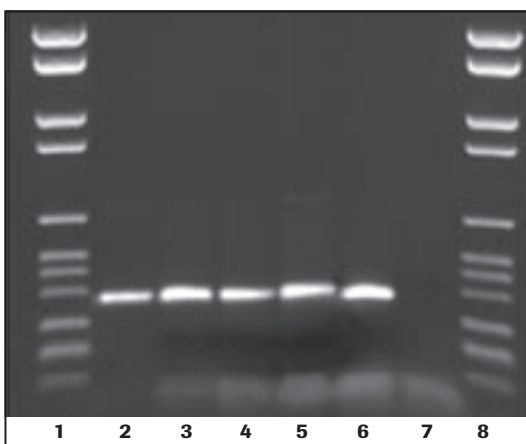


Figure 11: 1% agarose gel electrophoresis of 341 bp PCR product recovered in the presence of different amounts of Binding Enhancer.

A 341 bp PCR fragment of the tPA gene was amplified according to a standard block cycler protocol. Different amounts of BindingEnhancer were used in the PCR product purification procedure.

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

Figure 11 displays the efficient removal of small DNA fragments, especially in the absence of Binding Enhancer (Lane 2). This property of the kit is useful when DNA fragments are to be used in downstream applications like re-PCR, cloning or sequencing. For purification of small PCR fragments increasing binding enhancer concentrations are recommended for full recovery of all reaction products (Lane 3-5).

Product	Cat. No.	Pack Size
High Pure PCR	04 983 955 001	Up to 50
Cleanup Micro Kit	04 983 912 001	purifications Up to 200 purifications

Premium Post-PCR Performance

Flexibility unlimited

High Pure PCR Product Purification Kit

Choose the High Pure PCR Product Purification Kit for preparation of concentrated purified DNA which may be used directly for sequencing, cloning and other routine applications (Figure 12). Starting samples include DNA products from amplification reactions or cDNA synthesis, DNA from enzyme treatments (e.g., alkaline phosphatase, labeling, restriction digest) or agarose-gel slices, dilute nucleic acid solutions, or RNA from transcription reactions.

- **Save time.**
Multiple PCR products can be quickly purified in parallel in less than 10 minutes.
- **Enjoy efficient purification and downstream processing.**
Eliminate primers, nucleotides, salts, and contaminants that can reduce efficiency of cloning and other post-PCR applications.
- **Take advantage of a convenient kit and optimized protocol.**
Reduced handling steps minimize the loss and fragmentation of DNA.
- **Benefit from flexibility in choosing your starting sample.**
Use the same versatile kit to purify cDNA synthesis products, RNA from transcription reactions, and DNA from agarose-gel slices; additional purification options include concentration of dilute nucleic acid solutions and removal of labels from random-primed labeling reactions.

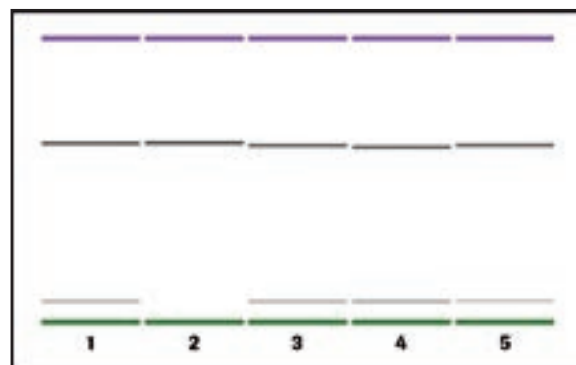


Figure 12: Capillary gel electrophoresis image of ds DNA fragments (50 bp & 400 bp) before and after purification using the High Pure PCR Product Purification Kit and products of other suppliers.

One and a half micro gram of a 50 bp and a 400 bp ds DNA fragment was mixed and purified using the High Pure PCR Product Purification Kit or products from other suppliers according to the provided instructions. After purification, samples were analyzed via capillary gel electrophoresis.

The purple and green bars indicates marker bands of the bio-analyzer instrument, the upper band indicates the 400 bp, the lower band the 50 bp ds DNA fragment.

Lane 1: Fragment mixture without purification

Lane 2: High Pure PCR Product Purification Kit

Lane 3: Supplier 1

Lane 4: Supplier 2

Lane 5: Supplier 3

Result: Reliable rapid purification of PCR products with high recovery and complete removal of low molecular weight material is only obtained with the High Pure PCR Product Purification Kit.

Product	Cat. No.	Pack Size
High Pure PCR Product Purification Kit	11 732 668 001	50 purifications
	11 732 676 001	250 purifications

High Pure 96 UF Cleanup Kit

Choose the High Pure 96 UF Cleanup Kit for efficient and reliable isolation of highly pure and concentrated PCR fragments for high-throughput applications, including fluorescent sequencing, labeling, cloning, restriction digest, and microarray spotting. Simply load, filter, suspend, and recover your purified PCR product.

The kit is based on ultrafiltration technology and incorporates the convenience of a 96-well format. Small contaminants (*e.g.*, dNTP'S, primers, primer-dimers, salts), which often interfere with downstream applications are quickly removed (Figure 13).

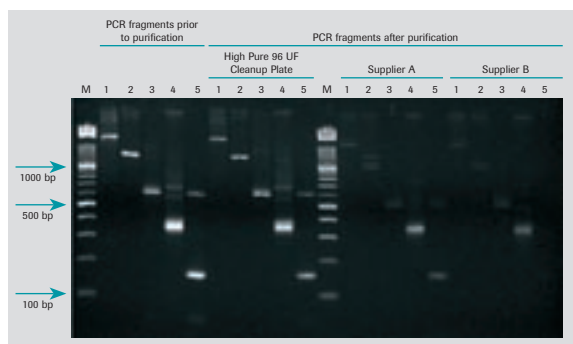


Figure 13: Isolation of PCR fragments. Equal amounts of sample were loaded per well after purification using the indicated products.

Lanes 1: 1.7-kb PCR fragment

Lanes 2: 1.2-kb PCR fragment

Lanes 3: 600-bp PCR fragment

Lanes 4: 350-bp PCR fragment

Lanes 5: 165-bp PCR fragment

M: DNA Molecular Weight Marker XIV

- **Choose a flexible format.**

Samples can be processed manually using vacuum manifolds or microplate centrifuges, or automatically with common liquid-handling instruments.

- **Reduce purification time.**

All reagents and plates are ready-to-use and supplied with the kit.

- **Benefit from a quick and simple procedure.**

Up to 96 samples can be processed in parallel in less than 20 minutes.

- **Insist on reliability.**

Obtain highly purified DNA fragments as small as 150 bp.

- **Easily recover DNA from the robust ultrafiltration membrane.**

Membrane parts do not interfere in microarray spotting.

- **Avoid cross contamination.**

96 individual columns help prevent well-to-well or aerosol crosstalk; samples are both applied and removed from the column top.

Product	Cat. No.	Pack Size
High Pure 96 UF Cleanup Kit	04 422 694 001	192 purifications

Premium Post-PCR Performance

Flexibility unlimited

Agarose Gel DNA Extraction Kit

Use this kit to efficiently purify specific DNA fragments from standard or low melting point agarose with excellent yield. The kit is an ideal tool for concentrating aqueous DNA solutions. The isolated DNA fragments are ready for use in subsequent reactions (e.g., ligation, enzymatic restriction, labeling, or sequencing). The purification process does not inhibit subsequent restriction enzyme digestion (Figure 14).

- **Benefit from a quick and simple procedure.**
Extract DNA in only 45 minutes, with few hands-on steps.
- **Use your choice of agaroses and buffer systems.**
Low melting point agarose is not required.
- **Purify efficiently.**
The highly specific binding of DNA allows easy removal of impurities.
- **Benefit from a gentle, high yield procedure.**
Shearing of large DNA fragments is prevented due to the uniformity and smooth surface of the silica particles.
- **Avoid enzymatic inhibition.**
The eluate is free of protein, small particles and other impurities which can interfere with subsequent reactions.

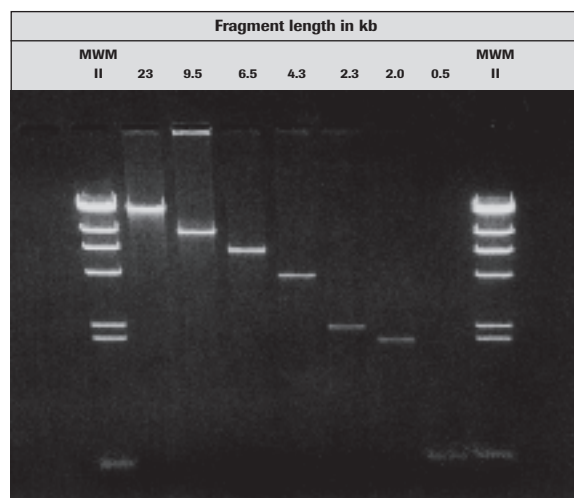


Figure 14: Recovery of DNA fragments from agarose gels. DNA fragments (0.5 – 23 kb) from the Roche Applied Science Molecular Weight Marker II (MWM II) were separated on a 1% agarose gel, extracted from the gel, and isolated according to the kit protocol. Isolated fragments were displayed on an agarose gel. The Agarose Gel DNA Extraction Kit isolated good yields of both large and small DNA fragments.

Product	Cat. No.	Pack Size
Agarose Gel DNA Extraction Kit	11 696 505 001	Up to 100 reactions

Resources

Nucleic Acid Isolation and Purification Manual, 3rd edition

Consult this manual to see how Roche Applied Science's nucleic acid preparation products can help you optimize your nucleic acid isolation results.

Included in the manual are:

- Step-by-step instructions
- Numerous tips for achieving optimal results
- A brief overview of commonly used methods
- Helpful hints and troubleshooting guidelines
- Details on innovative and optimized products for nucleic acid preparations

Rely on the manual's in-depth product description to guide you in selecting the ideal product for your application.

View the Nucleic Acid Isolation and Purification Manual online at www.roche-applied-science.com/ or request a printed version of the manual (cat. no. 05 092 434 001).



Support from the Web

For details on applications, protocols, products and manuals please visit our special interest site under:

<http://www.roche-applied-science.com/napure/>

Ordering Information

Product	Cat. No.	Pack Size
Agarose Gel DNA Extraction Kit	11 696 505 001	up to 100 purifications
DNA Isolation Kit for Cells and Tissues	11 814 770 001	10 isolations
DNA Isolation Kit for Mammalian Blood	11 667 327 001	25 purifications
Genopure Plasmid Midi Kit	03 143 414 001	up to 20 isolations
Genopure Plasmid Maxi Kit	03 143 422 001	up to 10 isolations
Genopure Buffer Set for Low-Copy Number Plasmids	04 634 772 001	1 set
High Pure FPPE RNA Micro Kit	04 823 125 001	up to 50 isolations
High Pure miRNA Isolation Kit	05 080 576 001	50 purifications
High Pure PCR Cleanup Micro Kit	04 983 955 001 04 983 912 001	50 purifications 200 purifications
High Pure PCR Product Purification Kit	11 732 668 001 11 732 676 001	50 purifications 250 purifications
High Pure PCR Template Preparation Kit	11 796 828 001	100 purifications
High Pure Plasmid Isolation Kit	11 754 777 001 11 754 785 001	50 purifications 250 purifications
High Pure RNA Tissue Kit	12 033 674 001	50 purifications
High Pure RNA Isolation Kit	11 828 665 001	50 purifications
High Pure RNA Paraffin Kit	03 270 289 001	100 isolations
High Pure Viral RNA Kit	11 858 882 001	100 purifications
High Pure Viral Nucleic Acid Kit	11 858 874 001	100 purifications
High Pure Viral Nucleic Acid Large Volume Kit	05 114 403 001	40 purifications
High Pure 16 System Viral Nucleic Acid Kit	12 011 816 001	6 x 16 isolations

Product	Cat. No.	Pack Size
High Pure 96 UF Cleanup Kit	04 422 694 001	2 x 96 purifications
Microarray Target Purification Kit	03 266 885 001	50 isolations
mRNA Capture Kit	11 787 896 001	192 reactions
mRNA Isolation Kit	11 741 985 001	>70 µg mRNA
mRNA Isolation Kit for Blood/Bone Marrow	11 934 333 001	30 - 100 isolations
mini Quick Spin DNA Columns	11 814 419 001	50 columns
mini Quick Spin Oligo Columns	11 814 397 001	50 columns
mini Quick Spin RNA Columns	11 814 427 001	50 columns
Quick Spin Columns for radiolabeled DNA purification, Sephadex G-25	11 273 922 001 11 273 949 001	20 columns 50 columns
Quick Spin Columns for radiolabeled DNA purification, Sephadex G-50	11 273 965 001 11 273 973 001	20 columns 50 columns
Quick Spin Columns for radiolabeled RNA purification, Sephadex G-25	11 273 990 001	20 columns
Quick Spin Columns for radiolabeled RNA purification, Sephadex G-50	11 274 015 001	20 columns
TriPure Isolation Reagent	11 667 157 001 11 667 165 001	50 ml 200 ml
Proteinase K recombinant PCR Grade (lyophilizate)	03 115 836 001 03 115 879 001 03 115 801 001 03 115 852 001	25 mg 100 mg 500 mg 1 g (4 x 250 mg)
Proteinase K recombinant, PCR Grade (solution)	03 115 887 001 03 115 828 001 03 115 844 001	1.25 ml 5 ml 25 ml
DNase I recombinant, RNase-free	04 716 728 001	10,000 units

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