Chromatography

Chromatography methods may utilize gel filtration, ion exchange, selective adsorption, or affinity binding.

Gel filtration exploits the molecular sieving properties of porous gel particles. A matrix with defined pore size allows smaller molecules to enter the pores by diffusion whereas bigger molecules are excluded from the pores and eluted at the void volume. Thus, molecules are eluted in order of decreasing molecular size.

Ion exchange chromatography depends on an electrostatic interaction between a target molecule and a functional group on the column matrix. The technique allows concentration and separation of molecules from a large volume in a short time. Nucleic acids – highly negatively charged, linear polyanions – can be eluted from ion exchange columns with simple salt buffers.

In *adsorption chromatography*, nucleic acids adsorb selectively onto silica or glass in the presence of chaotropic salts, while other biological molecules do not. A low salt buffer or water then elutes the nucleic acids, thereby producing a sample that could be used directly in most downstream applications.

Affinity chromatography is a highly specific adaptation of adsorption chromatography. An immobilized ligand recognizes and binds a particular structure on a biomolecule. Washes then remove unbound components (with different structures). Finally, a "competitor molecule" (which also recognizes the immobilized ligand) floods the binding sites on the affinity matrix, releasing the bound biomolecule.

Centrifugation

Selective centrifugation is a powerful purification method. For example, ultracentrifugation in self-forming CsCl gradients at high g-forces has long been used for plasmid purification.

Frequently, centrifugation is combined with another method. For example:

- Spin column chromatography combines gel filtration and centrifugation to purify DNA or RNA from smaller contaminants (salts, nucleotides, etc.), for buffer exchange or for size selection.
- Some procedures combine selective adsorption on a chromatographic matrix (see above) with centrifugal elution to selectively purify one type of nucleic acid.

Electrophoresis

Nucleic acids may be separated electrophoretically according to their size. This separation is most commonly done on agarose gels. In the presence of ethidium bromide, the separated nucleic acids may be seen under UV light.

Electrophoretic separation is also frequently used to determine size and purity of DNA. For example, after PCR, electrophoresis is used to quickly check product length and purity (absence of byproducts).

Affinity purification

In recent years, more and more purification methods have combined affinity immobilization of nucleic acids with magnetic separation. For instance, poly(A)$^+$ mRNA may be bound to streptavidin-coated magnetic particles by biotin-labeled oligo(dT) and the particle complex removed from the solution (and unbound contaminants) with a magnet. This solid phase technique simplifies nucleic acid purification, since it can replace several centrifugation, organic extraction, and phase separation steps with a single, rapid magnetic separation step.

Principle of silica adsorption

All the kits described in this chapter depend on the tendency of nucleic acids to adsorb to silica (glass) in the presence of a chaotropic salt such as sodium iodide (NaI), guanidine thiocyanate or guanidine hydrochloride (Melzak et al., 1996).

This tendency was discovered by Vogelstein and Gillespie (1979) who found that DNA fragments adsorbed to powdered flint glass in the presence of saturated NaI. Later work showed other nucleic acids adsorbed to glass in the presence of other chaotropes, including:

- DNA plasmids (Marko et al., 1982)
- Single-stranded phage nucleic acids (Kristensen et al., 1987; Zimmermann et al., 1989)
- Genomic DNA (Yamada et al., 1990; Zeillenger et al., 1993)
- Total RNA (Yamada et al., 1990)
- Rapid and simple method for purification of nucleic acids (Boom et al., 1990)

Different types of nucleic acid adsorb more or less tightly to glass depending on the ionic strength and the pH of the surrounding solution. A low salt buffer or water is always used to elute the nucleic acid from the glass. In each kit, this method is optimized to prepare a particular type of nucleic acid.

Overview of the procedure

All the High Pure kits use glass fiber fleece immobilized in a special plastic Filter Tube. The glass fleece filter:

- Adsorbs only nucleic acids, ensuring separation of the target molecules from a complex biological mixture of proteins, sugars, lipids and other components
- Can be inverted into a microcentrifuge tube and processed in a standard tabletop microcentrifuge
- Allows processing of 0.2 – 2.5 ml samples in a series of centrifugation steps
- Is specially constructed to ensure that contaminants suspended in the Wash Buffer are not retained by the filter or transferred to the eluted, purified nucleic acid

Benefit from smart column design

The High Pure Micro Column

- **Achieve high purity.** Novel optimized columns produce highly concentrated (10 µl) eluate and high recovery rates (>80%) of even small DNA and RNA fragments.
- **Up to 10 µg binding capacity** for use in demanding downstream applications.
- **Avoid carryover contamination** using a column design without a cavity for liquid transfer.

The High Pure Mini Column

- **Perform long template applications.** Efficiently purify high molecular weight DNA (30-50 kb).
- **Maximize performance and accuracy in downstream assays.** Achieve high sensitivity and reproducibility in many applications with a binding capacity of up to 100 µg.
- **Obtain accurate results.** Use highly pure, concentrated (50 µl) nucleic acids in real-time PCR and other applications.

2

The High Pure Extender Assembly

- **Benefit from smart column design** using a removable High Pure mini spin column.
- **Improve sensitivity.** Use sample volumes up to 2.5 ml for high yields of purified nucleic acids concentrated in 50 µl.
- **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.

In each High Pure kit, the steps are basically the same and require only a few minutes. The nucleic acids prepared with the High Pure kits may be used directly in a variety of down-stream applications.

Substance added to serum	Highest concentration tested with no inhibition in PCR after High Pure purification
Citrate	30 mg/ml
EDTA	300 mg/ml
Heparin	30 U/ml
Hemoglobin	50 mg/ml

Performance of High Pure Nucleic Acid Purification in the removal of different anticoagulants or human hemoglobin. The table indicates the highest concentration tested which showed no inhibition.

Instead of glass fiber fleece (as in the High Pure kits), one can also use silica beads to adsorb DNA.

- The Agarose Gel DNA Extraction Kit starts with an agarose gel slice containing a DNA fragment, then solubilizes that gel and releases the DNA into the starting solution

The kit uses steps similar to those of the High Pure kits to purify the DNA from the starting material. In each, the nucleic acid is adsorbed to silica in the presence of a chaotropic salt, collected by centrifugation (while adsorbed to the silica beads), washed extensively to remove contaminants, then released from the beads with a low salt buffer.

The DNA isolated with the kit is pure enough to be used directly in labeling, sequencing, cloning, and other procedures that require concentrated DNA.

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2

Experiment 3

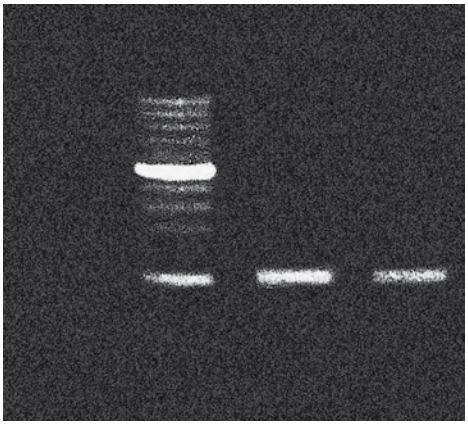


Figure 3: Use of nucleic acids from paraffin-embedded tissue (prepared with the High Pure PCR Template Preparation Kit) for amplification of the human androgen receptor gene. Nucleic acids were purified from formalin-fixed, paraffin-embedded samples of human prostate tissue by Protocol Ve. An aliquot (10 µl) of each nucleic acid product was used as template for the Expand High Fidelity PCR System (100 µl reaction mixture). The primers were derived from exon 7 of the androgen receptor gene. Aliquots (20 µl) of each amplicon were separated electrophoretically and the gel was stained with ethidium bromide.

Lane 1: Molecular Weight Marker VIII
Lane 2: Androgen receptor amplicon from one tissue section
Lane 3: Androgen receptor amplicon from half a tissue section

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

Experiment 4

	Incubation period		
	15 min	30 min	60 min
L. casei			
Without Mu	15.2 (40.0 %)	21.0 (53.2 %)	21.8 (57.4 %)
0.25 U/µl Mu	15.3 (40.3 %)	24.6 (64.7 %)	24.0 (63.2 %)
0.5 U/µl Mu	16.4 (43.2 %)	38.0 (100 %)	30.4 (80.0 %)
L. curvatus			
Without Mu	9.2 (10.6 %)	14.7 (17.0 %)	26.1 (30.2 %)
0.25 U/µl Mu	25.9 (29.9 %)	61.3 (70.9 %)	70.7 (81.7 %)
0.5 U/µl Mu	46.0 (53.2 %)	86.5 (100 %)	131.1 (151.6 %)
L. sakei			
Without Mu	13.5 (11.4 %)	16.6 (14.0 %)	52.9 (44.7 %)
0.25 U/µl Mu	35.0 (29.6 %)	71.8 (60.6 %)	168.2 (142.1 %)
0.5 U/µl Mu	79.3 (67.0 %)	118.4 (100 %)	196.1 (165.6 %)
S. aureus			
Without Ly	10.3 (10.8 %)	30.5 (31.9 %)	64.2 (67.2 %)
10 µg/ml Ly	16.5 (17.3 %)	95.0 (99.5 %)	105.2 (110.2 %)
25 µg/ml Ly	16.2 (17.0 %)	95.5 (100 %)	106.7 (111.7 %)

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

2

LightCycler® sample preparation

The High Pure PCR Template Preparation Kit has been evaluated for LightCycler® sample preparation with whole blood and cultured cells as sample material. Amplification has been performed in LightCycler® capillaries using SYBR Green and Hybridization Probes as the detection formats. The following tables give information about the range of sample volume applied, modifications in the sample preparation procedure and expected results.

Sample	Volume/Amount Range	Typical concentration range [ng/µl]	Eluate use in LightCycler® PCR (µl)
Whole blood human*	1 – 50 µl **	0.5 – 25	1 – 5 [0.5 ng – 125 ng]
Cultured cells, K562*	100 – 10^5 cells	0.01 – 10	1 – 5 [0.01 – 50 ng]

* Research samples
** Yield may vary between different blood donors because they may have different amounts of leukocytes

Procedure modification

The standard procedure for whole blood and cultured cells has been used for LightCycler® sample preparation except the elution volume was set to 50 µl in order to increase nucleic acid concentration for minute sample amounts. For larger sample amounts the standard elution volume of 200 µl can be applied.

Parameter	Sample Material	SYBR Green		HybProbes	
		Min	Max	Min	Max
cyclophilin A	blood	0.005 µl[a)]	100 µl	0.005 µl[a)]	100 µl
or β-globin	cells	1[a)]	10^4	1[a)]	10^5

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

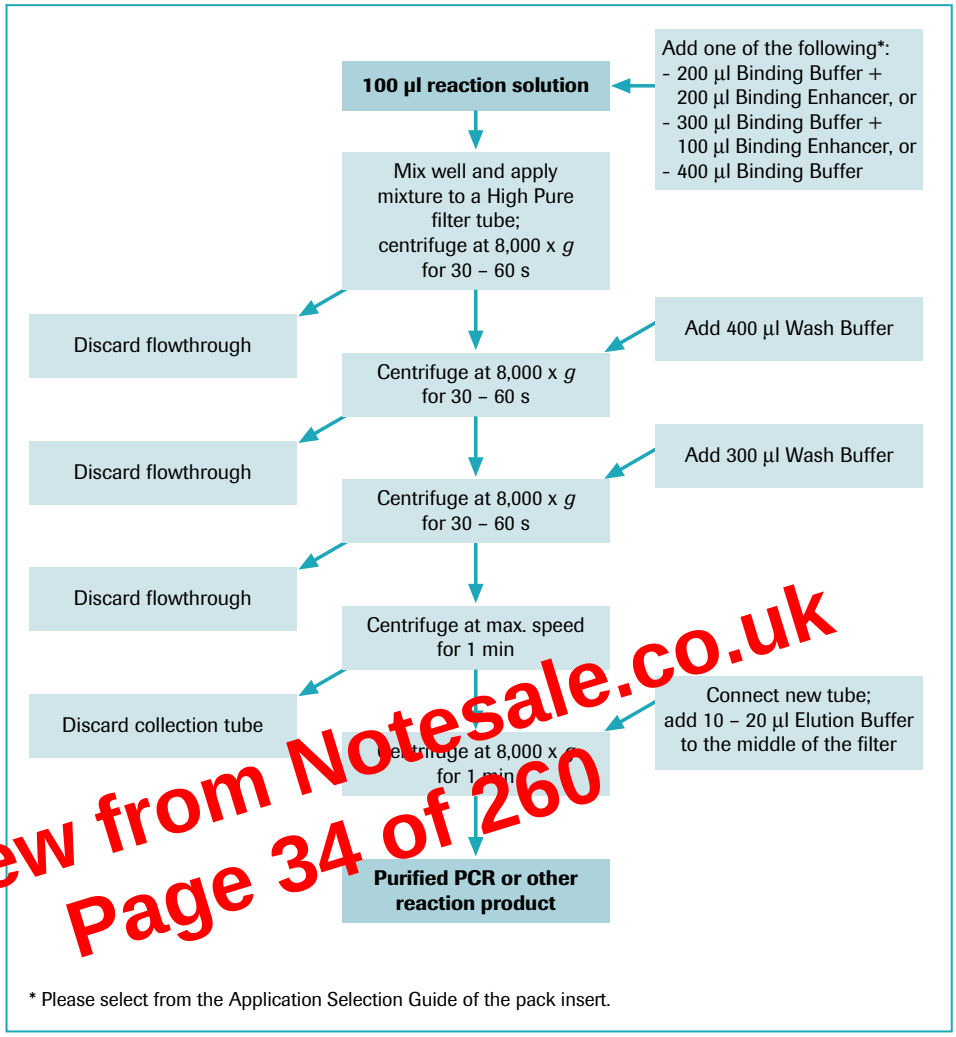
a) The used quantities of eluate correspond to these calculated amounts.

Time required	The entire High Pure PCR Cleanup Micro Kit method takes approx. 10 min.
Results	The amount of DNA recovered is dependent on the amount of DNA applied to the glass fiber fleece, the elution volume, and the length of the amplification/DNA products. When 5 - 25 µg DNA is applied to the kit's High Pure Micro Filter Tube, approximately 80% of the DNA can be recovered.
Benefits	• Conserve resources by using one versatile kit that eliminates the need to use several kits from other suppliers. • Save time with a simple and rapid protocol that reduces purification time. • Obtain purified product in a small elution volume (≤10 µl) for demanding down-stream applications. • Efficiently remove contaminants and unwanted reaction components. • Generate contaminant-free DNA for direct use in cloning, ligation, restriction digests, and other reactions. • Selectively isolate specific DNA fragment sizes by using the kit's binding enhancer to adjust purification stringency.

2

How to use the kit

I. Flow diagram



* Please select from the Application Selection Guide of the pack insert.

II. Kit contents

- Binding Buffer 20 ml or 80 ml
- Binding Enhancer 15 ml or 45 ml
- Wash Buffer 10 ml or 2 x 20 ml
- Elution Buffer 40 ml
- High Pure Micro Filter Tubes (50 or 200 tubes)
- Collection Tubes (50 or 200 tubes)

2

Vc. Purifying labeled probes

1. Fill up the labeling reaction to 100 µl with PCR grade water.
2. Add 500 µl Binding Buffer and mix well.

   It is important that the volume ratio between sample and Binding Buffer is 1:5. When using other sample volumes than 100 µl, adjust the volume of Binding Buffer accordingly.
3. Follow Protocol Va starting at the sample transfer to the Filter Tube assembly (Step 2).

   The elution efficiency is increased with higher volume of Elution Buffer applied. At least 68% and 79% recovery are found with 50 and 100 µl Elution Buffer, respectively. Normally, almost quantitative recovery can be found, as can be determined in a direct detection assay.

VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure PCR Product Purification Kit, see page 116.

Typical results with the kit

Experiment 1

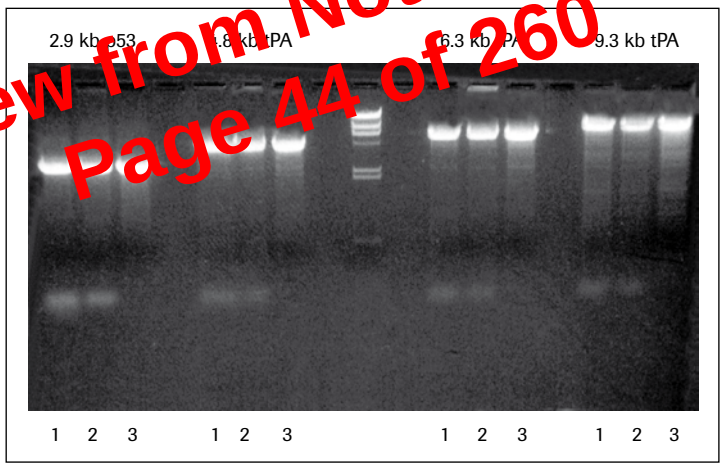


Figure 8: Comparison of PCR products purified with the High Pure PCR Product Purification Kit and those purified by phenol/chloroform extraction.
Four different long PCR products were generated with the Expand Long Template PCR System, purified by either of two methods, then analyzed electrophoretically. From left to right, the four products shown on the gel are: a 2.9 kb fragment from the p53 gene; 4.8 kb, 6.3 kb, and 9.3 kb fragments from the tissue plasminogen activator (tPA) gene. For each size product, the gel shows:

Lane 1: DNA before purification
Lane 2: DNA after purification by phenol/chloroform extraction
Lane 3: DNA after purification with the High Pure PCR Product Purification Kit

Result: The High Pure Kit removed primers and primer dimers, while recovering 90% of the long PCR products (as calculated by photospectroscopy).

2

Experiment 2

Preparation of hybridization probes for the analysis of number and expression patterns from sulfurtransferases from Arabidopsis

(kindly provided by J. Papenbrock, P. von Trzebiatowski and A. Schmidt, University of Hannover, Germany)

Background: Sulfurtransferases are a group of enzymes widely distributed in plants, animals, and bacteria. They catalyze the transfer of a sulfane atom from a donor molecule to a thiophilic acceptor substrate.

In a database search several *Arabidopsis* EST clones with homologies to bacterial and mammalian sulfurtransferases could be identified. One of them was used to isolate a full length clone by screening an *Arabidopsis* cDNA library. The protein was expressed in *E. coli* and was determined to be a sulfurtransferase with high specificity for 3-mercaptopyruvate. However, RNA expression data, protein contents obtained by Western blot analysis and sulfurtransferase enzyme activity measurements using 3-mercaptopyruvate or thiosulfate as substrates did not correlate with each other in *Arabidopsis* plants in different developmental states. Therefore, we hypothesize the existence of further sulfurtransferases in *Arabidopsis*. Different EST clones that show homology to sulfurtransferases and also to senescence-associated proteins are initially characterized.

Methods: Genomic Southern blot was performed as previously described. For preparing the hybridization probes cDNAs of interest were separated from the vectors with the respective restriction enzymes (MST1 coding for the mature 3-mercaptopyruvate sulfurtransferase, 950 bp, *Kpn* I/Pst I; EST 46D8T7, 600 bp, *Sal* I/ *Not* I). DNA fragments were separated by agarose gel electrophoresis. The gel pieces containing the MST1 or the 46D8T7 fragment were eluted following exactly the inserted recipe for isolating DNA from agarose gels using the High Pure PCR Product Purification Kit. Eluted DNA was used for random prime labeling.

Northern blot was performed as previously described. The PCR probe used in Northern blotting was purified in the following way: The gel piece of interest was excised and the labeled fragment was purified with the High Pure PCR Product Purification Kit according to the inserted recipe for isolating DNA from agarose gels with an elution volume of 200 µl.

Results: *Hybridization of restricted genomic Arabidopsis DNA with a digoxigenin-labeled MST1 probe by random priming reveals the existence of at least two, and probably three different sulfurtransferase genes in the Arabidopsis genome (Figure 9, right). The pattern is similar to the hybridization results using the 46D8T7 cDNA but not identical (Figure 9, left).*

RNA homologous to the sequence of EST clone 46D8T7 was expressed in very young plants. The expression level decreases drastically and increases continuously during aging (Figure 10). In the plants of a later developmental stage, expression level parallels the expression of SAG 13 clone, the best marker of senescence known so far.

2

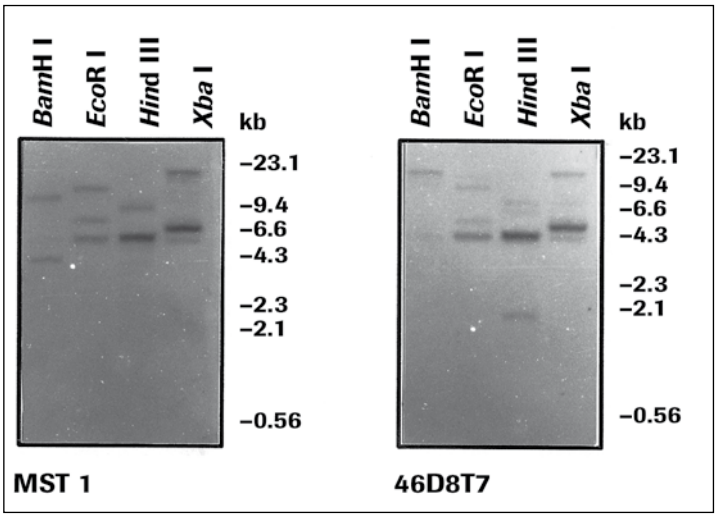


Figure 9: Southern blot analysis. Genomic DNA was restricted with BamH I, EcoR I, Hind III, and Xba I overnight, transferred to nylon membrane and hybridized with a digoxigenin probe labeled by random prime labeling.

Left: MST1 (3-mercapto-pyruvate sulfurtransferase) cDNA was used as a probe.

Right: cDNA from the EST clone 46D8T7 was labeled.

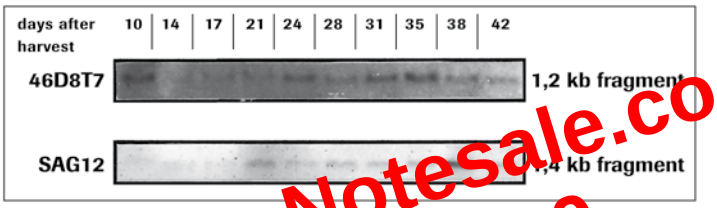


Figure 10: Northern blot analysis. Total RNA was extracted from Arabidopsis plants of different ages (from the left to the right harvested after 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 days), separated under denaturing conditions and transferred to nylon membranes. Equal loading was controlled by ethidium bromide staining. Filters were hybridized with PCR products labeled with digoxigenin. PCR-probe (600 bp) used for hybridizing the filter at the top: cDNA from EST clone 46D8T7. PCR-probe (1300 bp) used for hybridizing the filter at the bottom: cDNA from the SAG13 clone (senescence associated gene).

Discussion: The use of digoxigenin-labeled probes leads to very sharp bands in genomic Southern blotting. Purification of digoxigenin-labeled DNA via PCR using the High Pure PCR Product Purification Kit seems to increase the sensitivity of probes and reduce unspecific binding.

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High Pure RNA Isolation Kit

for isolation of total RNA from up to 50 samples

Cat. No. 11 828 665 001

Principle

A single reagent lyses the sample and inactivates RNase. In the presence of a chaotropic salt (guanidine HCl), the released total RNA binds selectively to glass fiber fleece in a special centrifuge tube. The RNA remains bound while a DNase treatment and a series of rapid "wash-and-spin" steps remove DNA and contaminating small molecules. Finally, low salt elution removes the RNA from the glass fiber fleece. The process does not require precipitation, organic solvent extractions, or extensive handling of the RNA.

Starting material

- 10^6 cultured mammalian cells (research samples)
- 200 – 500 µl human whole blood (research samples), containing anticoagulant
- 10^8 yeast (mid- to late-log phase, A_{600} <2.0)
- 10^9 gram positive or gram negative bacteria

Application

- Preparation of intact total RNA, which may be used directly in RT-PCR, Differential Display RT-PCR (DDRT-PCR), Northern blotting, primer extension assays, RACE (rapid amplification of cDNA ends), cDNA library construction, *in vitro* translation, or nuclease protection assays.

  RNA prepared from human whole blood is suitable for RT-PCR only.

Time required

- Total time: approx. 25 min (+ sample pretreatment, if needed)
- Hands-on time: <10 min (+ sample pretreatment, if needed)

Results

- Yield: Variable, depending on sample type (See the table under Part IV of "How to use the kit" in this article).
- Purity: Purified RNA is free of DNA, protein, salts, and other cellular components.

Benefits

- **Saves time**, because the kit can produce multiple RNA samples in minutes.
- **Ideal for a wide variety of samples**, because one kit can purify RNA from many sources.
- **Minimizes RNA loss**, because the kit removes contaminants without time-consuming precipitation or solvent extraction.
- **Increases lab safety**, because the kit does not use hazardous organic solvents.

2

Follow protocol Va starting with the DNase incubation (Step 5).

Vc. Isolation of total RNA from 10^8 yeast (S. cerevisiae)

Use fresh yeast that were harvested during mid- or late-log phase (A_{600} <2.0).

1. For each sample (10^8 yeast):
   - Collect the yeast by centrifugation at 2000 x *g* for 5 min in a standard tabletop centrifuge.
   - Resuspend the pellet in 200 µl of PBS and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.
2. Add 10 µl lyticase solution (0.5 mg/ml) to each microcentrifuge tube. Incubate the tube for 15 min at +30°C.
3. Follow Protocol Va above, starting with the Lysis/Binding Buffer step (Step 2).

Vd. Isolation of total RNA from 10^9 bacteria (gram positive or gram negative)

1. For each sample (10^9 bacteria):
   - Collect the bacteria by centrifugation at approximately 2000 x *g* for 5 min in a standard tabletop centrifuge.
   - Resuspend the pellet in 200 µl of 10 mM Tris-HCl, pH 8.0, and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.
2. Add 4 µl lysozyme solution (50 mg/ml in Tris-HCl, pH 8.0) to each microcentrifuge tube. Incubate the tube for 10 min at +37°C.
3. Add 400 µl Lysis/Binding Buffer to the sample and mix well.
4. To transfer the sample to a High Pure Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipet the entire sample into upper buffer reservoir of the Filter Tube.
5. Centrifuge the High Pure Tube assembly as in Step 4, Protocol Va.
6. Prepare DNase solution as in Step 5, Protocol Va. After centrifugation:
   - Add 100 µl DNase solution to the upper reservoir of the Filter Tube.
   - Incubate the Filter Tube for 60 min at +15 to +25°C.
7. Follow Protocol Va above, starting with the first wash (Step 6).

VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure RNA Isolation Kit, see page 117.

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⑬ The microcentrifuge tube now contains the eluted RNA. Either use 0.5 – 5 µl of the eluted RNA directly in RT-PCR or store the eluted RNA at –80°C for later analysis.

Before photometric determination of the RNA concentration, centrifuge the eluate for 2 min at maximum speed and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocols, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure FFPE RNA Micro Kit, see page 119.

2

Typical results with the kit

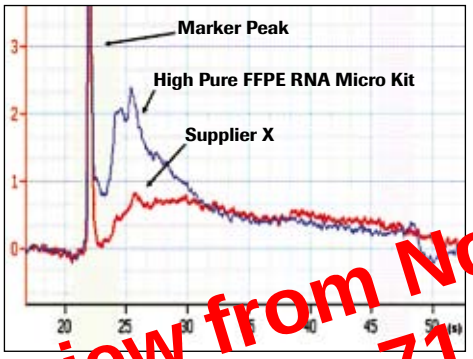


Figure 19: Fragment-length distribution of isolated RNA. RNA was isolated from a 5 µm section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The size distribution of the recovered RNA fragments was determined on an electropherogram (Bioanalyzer, Agilent).

Result: The High Pure FFPE RNA Micro Kit recovers optimal amounts of the different RNA fragments in the sample, even the small ones.

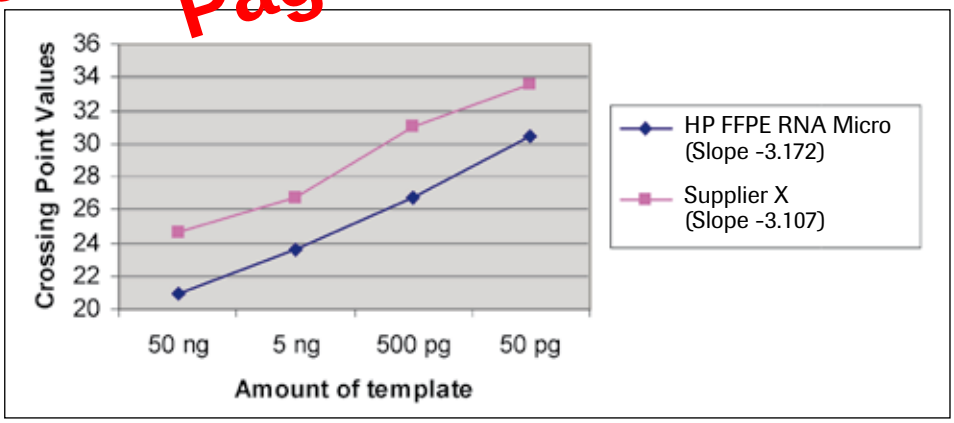


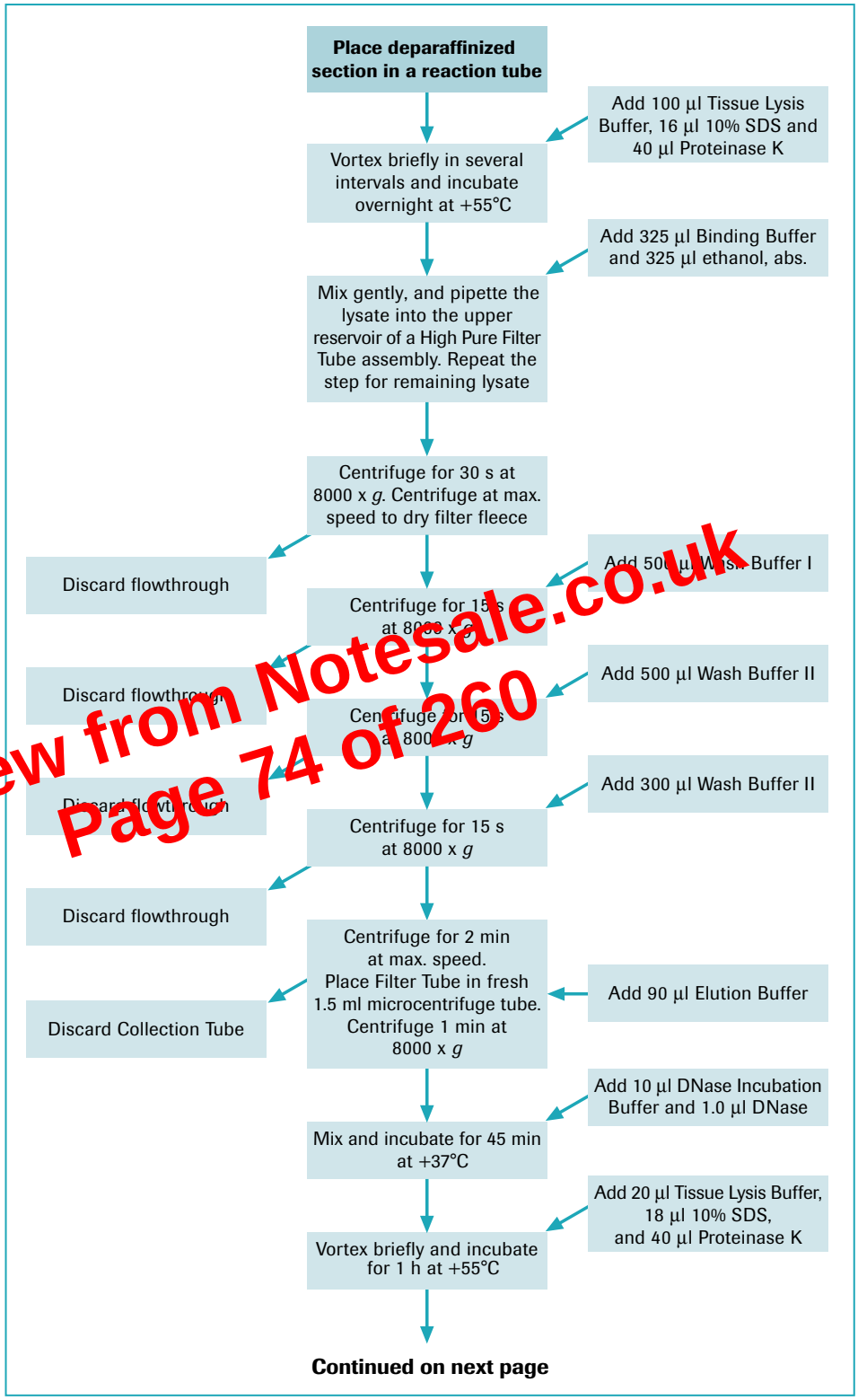
Figure 20: Performance of isolated RNA in RT-PCR. RNA was isolated from a 5 µm section of an FFPE breast tumor research sample, using either the High Pure FFPE RNA Micro Kit or a kit from another manufacturer (Supplier X). The isolated RNA samples were serially diluted and used as templates in separate RT-PCRs. A β-2microtubulin-specific amplification was performed, using the LightCycler® 1.5 Instrument and LightCycler® RNA Amplification Kit SYBR Green I.

Result: Template RNA isolated with the High Pure FFPE RNA Micro Kit performs well in the RT-PCR, giving linear results (based on the consistent slope obtained with serial dilutions) and high sensitivity (based on the early crossing points observed).

2

How to use the kit

I. Flow diagram



Vc. Protocol for the optional treatment of the eluate with DNase

For DNase treatment it is recommended to elute the sample with 100 µl Elution Buffer to minimize loss of sample material.

1. Preparation of DNase Solution:
   - For 70 µl DNase solution mix 49.5 µl PCR grade water, 19.5 µl 10 × DNase Incubation buffer and 1 µl DNase 1 (10 U/µl)
     DNase treatment on the column is not recommended as miRNA will elute at low salt concentrations.
2. Preparation of the reaction mix:
   - To approx. 100 µl eluate add 56 µl DNase Solution.
   - Vortex 3 × 5 s.
   - Incubate for 30 min at +37°C.
3. For reaction cleanup:
   - Add 325 µl Binding Buffer.
   - Vortex briefly.
   - Add 210 µl Binding Enhancer, vortex 3 × 5 s.
4. Combine the High Pure Filter Tube with a 2 ml collection tube and pipette the DNase treated sample into the upper reservoir.
   - Centrifuge for 30 s at 13,000 × *g* in a microcentrifuge and discard the flowthrough.
5. Prepare the first wash:
   - Add 500 µl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × *g*, discard the flowthrough.
6. After the first wash:
   - Add 300 µl Wash Buffer working solution.
   - Centrifuge for 30 s at 13,000 × *g*, discard the flowthrough.
7. To dry the glass fiber fleece:
   - Centrifuge at 13,000 × *g* for 1 min, in order to dry the filter fleece completely.
8. To elute the RNA:
   - Place the High Pure filter tube in a fresh 1.5 ml microcentrifuge tube, add 100 µl Elution Buffer and incubate for 1 min at +15 to +25°C.
     To increase RNA concentration of your sample, elution with 50 µl Elution Buffer is possible. An elution step with 100 µl Elution Buffer will increase the total yield by approx. 10 %.
   - Centrifuge for 1 min at 13,000 × *g*.
     The microcentrifuge tube now contains the eluted microRNA. Either use 0.5 – 5 µl of the eluted RNA directly in RT-PCR or store the eluted microRNA at –80°C for later analysis.
     Glass fibers in the eluate may interfere with optical density measurement. Before determining the RNA concentration photometrically, centrifuge and transfer supernatant to a fresh 1.5 ml reaction tube.

VI. Troubleshooting the High Pure Protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual.

2

Typical results with the kit

Experiment 1

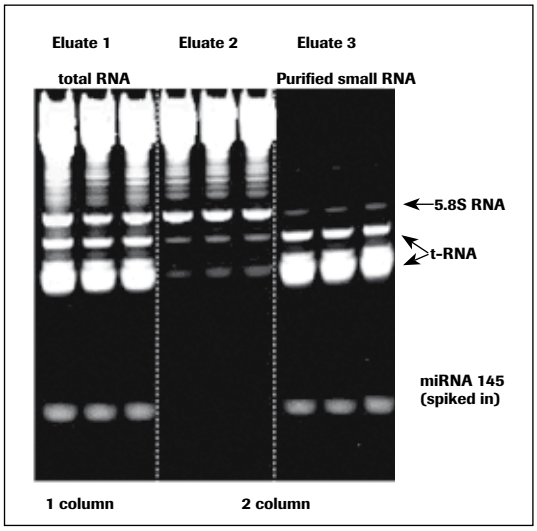
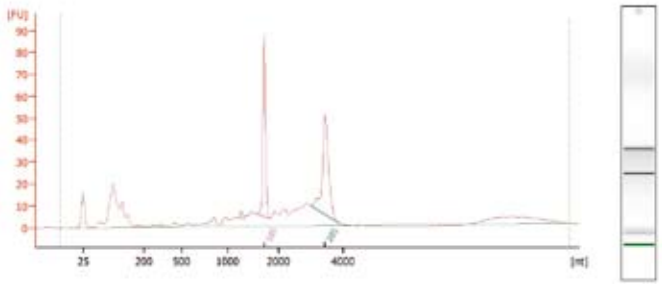


Figure 23: The integrity and size distribution of total RNA and small RNA (2-column protocol) purified with the High Pure miRNA Isolation Kit have been checked electrophoretically on a denaturing gel (15% acrylamide/TBE/urea). A 1 µg sample of miRNA 145 was spiked into a liver tissue lysate before purification. Nucleic acids were visualized by ethidium bromide staining.
Alternatively, electropherograms were recorded on an Agilent Bioanalyzer (Fig 20).

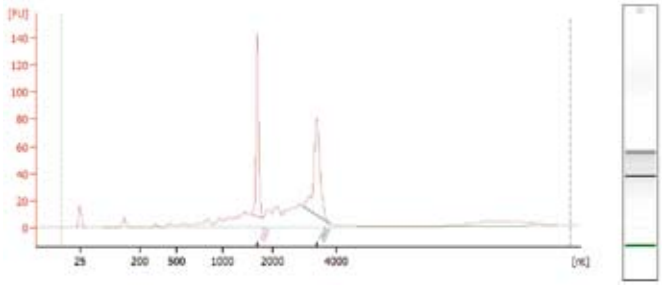
Eluate volume: 100 µl; 10 µl sample per lane
Eluate 1: 1-Column protocol; total RNA
Eluate 2: 2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification)
Eluate 3: 2-Column protocol; purified small RNA (miRNA enriched)

Result: Purified small RNA (2-column protocol) is free of DNA, nucleases and all cellular and sample contaminants that interfere with RT-PCR. The absence of contaminating DNA is examined by PCR without a preceding RT-reaction. No amplification product is obtained (data not shown).

1-Column protocol; total RNA from 5 mg liver (mouse) stabilized in RNAlater®



2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification) from 5 mg liver (mouse) stabilized in RNAlater®



2-Column protocol; purified small RNA (miRNA enriched) from 5 mg liver (mouse) stabilized in RNAlater®

Figure 24: Electropherograms recorded on an Agilent Bioanalyzer
Eluate 1: 1-Column protocol; total RNA
Eluate 2: 2-Column protocol; high molecular weight RNA eluted from the first filter tube (control of purification)
Eluate 3: 2-Column protocol; purified small RNA (miRNA enriched)

10 The microcentrifuge tube now contains the eluted viral RNA. You may:

- EITHER use an aliquot of the eluted RNA directly in RT-PCR

  Use 3.5 – 6 µl of the eluate for the reverse transcriptase reaction.

- OR store the eluted RNA for later analysis at –80°C.

V. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 113 of this manual. For factors that may affect the High Pure Viral RNA Kit, see page 120.

Typical result with the kit

The kit was used to prepare genomic RNA from viruses [for example, hepatitis C virus (HCV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV)] for research applications. Each preparation was used as a template in RT-PCR. All these templates produced highly specific PCR products in good yield.

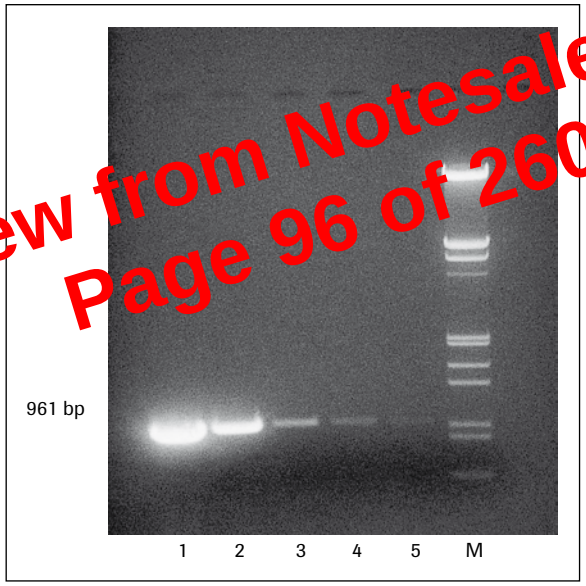


Figure 2.6: RT-PCR analysis of MS2 RNA isolated with the High Pure Viral RNA Kit. Serial dilutions of purified MS2 RNA were applied to the Filter Tubes, washed and eluted following the kit protocol. 3.5 µl of the 50 µl eluate were analyzed by two step RT-PCR using primers that resulted in a fragment of 961 bp. The indicated numbers of molecules per PCR correspond to assumed quantitative recovery.

Lane 1: 3.5 x 10^7 molecules/PCR;
Lane 2: 3.5 x 10^5 molecules/PCR;
Lane 3: 3.5 x 10^3 molecules/PCR
Lane 4: 3.5 x 10^2 molecules/PCR
Lane 5: 35 molecules/PCR
Lane M: DNA Molecular Weight Marker III

Result: Even with theoretically 35 molecules recovered, a detectable signal in agarose gel electrophoresis is obtained.

2

How to use the High Pure Extender Assembly

The High Pure Extender Assembly is delivered in single zip-bags. Five High Pure Extender Assemblies are additionally packed in labeled zip-bags.
Each High Pure Extender is assembled in a 50 ml polypropylene Tube.
The High Pure Extender Assembly is designed for use with table-top centrifuges and swing-bucket rotors with 4,000 x *g* force applicable.

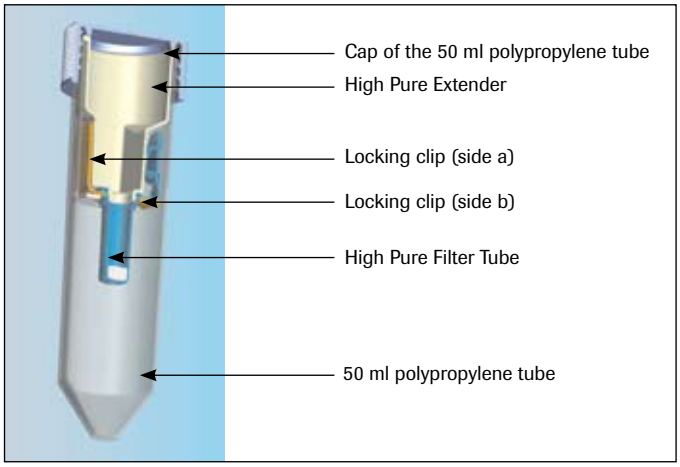


Figure 29: High Pure Extender Assembly
Remove the High Pure Extender Assembly from the zip-bags prior to use. In order to load the sample onto the Assembly unscrew the cap of the 50 ml polypropylene tube. After sample loading, close the High Pure Extender Assembly with the 50 ml polypropylene tube cap.
After the first centrifugation step, the sample has passed through the High Pure Extender Assembly and is collected at the bottom of the 50 ml polypropylene tube. Nucleic acids are bound to the silica fleece at the bottom of the High Pure filter tube. For further processing, remove the High Pure Extender Assembly from the 50 ml polypropylene tube. Discard the tube containing the flow-through. Remove the High Pure Filter Tube from the High Pure Extender Assembly (see Figures 31 to 33).

Disassembly of the High Pure Extender Assembly

Figure 30: Unscrew the 50 ml polypropylene tube and remove the High Pure Extender Assembly from the 50 ml polypropylene tube.

Figure 31: Place the High Pure Extender in a new collection tube, which is placed securely in a tube rack on the bench.

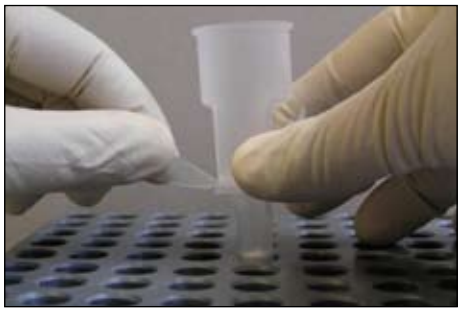


Figure 32: Secure the High Pure Extender Assembly with one hand while grasping the locking clip (side a) of the High Pure Filter Tube cap on the opposite side with the other hand. Remove the first part of the locking clip (side a) by screwing the clip in either direction.

Figure 33: Rotate the High Pure Extender Assembly. Remove the second part of the locking clip (side b) by pulling the locking clip away from the Extender Assembly.

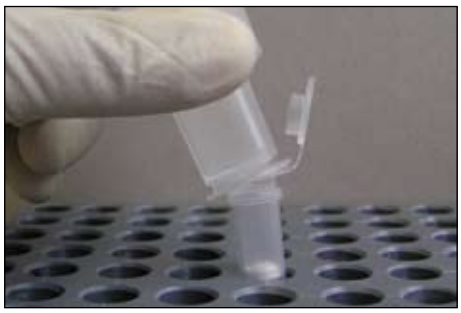


Figure 34: Remove the High Pure Extender from the High Pure Filter Tube by tilting the High Pure Extender away from the High Pure Filter tube toward the side without the cap.

We do not recommend the use of fixed-angle rotors in combination with the High Pure Extender Assembly.
However, if you decide to use a centrifuge with fixed-angle rotors, do not centrifuge High Pure Extender Assembly above 3000 × g. The use of fixed-angle rotors results in incomplete flow of the liquid through the Assembly. The remaining sample solution therefore stays within the High Pure Spin Column Filter Tube. Remove this remaining liquid by an additional spin of the High Pure Filter Tube in a bench-top centrifuge before the first washing step. For this additional spin remove the High Pure Spin Column from the High Pure Extender Assembly according to Figures 30 to 35.

Both kits are supplied with folded filters to eliminate the time-consuming centrifugation step after the alkaline lysis. In approximately 2 min (midi) or 10 min (maxi), respectively, of unattended running cellular debris and potassium dodecyl sulfate precipitates are held back by the filter thereby avoiding shearing of large DNA constructs. Besides the significant reduction of preparation time another advantage of filtration is that even small SDS precipitates which cannot be separated by conventional centrifugation are completely removed.

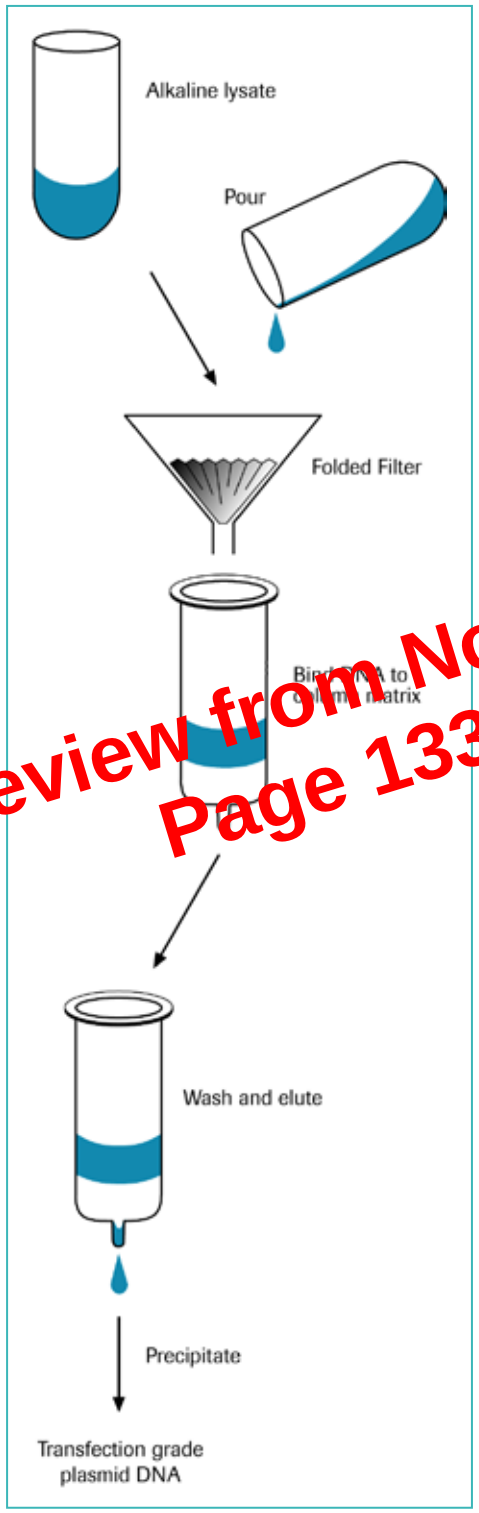


Figure 40: Genopure plasmid purification procedures.
Schematic representation of clearing the lysate by filtration with subsequent plasmid purification.

II. Kit contents

- Suspension Buffer (150 ml) for suspension of bacterial cell pellets
- RNase A (15 mg) for dissolution in Suspension Buffer
- Lysis Buffer (150 ml) for bacterial cell lysis
- Neutralization Buffer (150 ml) to form a stable cellular debris precipitate
- Equilibration Buffer (70 ml) for equilibrating the columns prior to use
- Wash Buffer (370 ml) for removal of residual impurities
- Elution buffer (200 ml) for plasmid elution
- NucleoBond AX 500 Columns (10 columns) for the isolation step
- Folded filters (10 filters) to eliminate a centrifugation step and to remove cellular debris
- Sealing rings (5 rings) to station the columns in test tubes

III. Additional materials needed

- Centrifuge and tubes for harvesting bacterial cultures, capable of ≥15,000 x *g*
- Isopropanol
- 70% ethanol
- TE buffer or other low salt buffer
- Tube for collecting and precipitating filtered plasmid DNA
- Funnel for clearing of lysates by folded filters

IV. Protocol for high copy number plasmid DNA, continued

Step	Action	Time / x *g* / Temperature
9	▶ Re-insert the column into a new Collection Tube capable of withstanding high speed centrifugation (≥15,000 x *g*).	
	▶ Elute the plasmid with 15 ml Elution Buffer.	
	▶ Allow the column to empty by gravity flow.	
	▶ The collected flowthrough contains the plasmid.	
10	▶ Precipitate the eluted plasmid DNA with 11 ml isopropanol equilibrated to +15 to +25°C.	
	▶ Centrifuge immediately at high speed.	30 min / ≥15,000 x *g* / +2 to +8°C
	▶ Carefully discard the supernatant.	
11	▶ Wash the plasmid DNA with 4 ml chilled 70% ethanol.	+2 to +8°C
	▶ Centrifuge at high speed.	10 min / >15,000 x *g* / +2 to +8°C
	▶ Carefully remove ethanol from the tube with pipette tip.	
	▶ Air-dry the plasmid DNA pellet.	10 min
12	▶ Carefully redissolve the plasmid DNA pellet in 100 – 500 µl TE-buffer or PCR grade water.	

V. Troubleshooting the Genopure Plasmid Maxi protocol

If you get...	Then, the cause may be...	And you should...
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	▶ Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness	▶ Store all buffers at +15 to +25°C. ▶ After reconstitution of RNase with Suspension Buffer store aliquots at +2 to +8°C. ▶ Close all reagent bottles tightly after each use to preserve pH, stability and freedom from contamination.
	Reagents and samples not completely mixed	▶ Always mix the sample tube well after addition of each reagent. ▶ Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.
Low recovery of nucleic acids after elution	Non-optimal reagent has been used for elution. Salt is required for optimal elution	▶ Use the Elution Buffer of the kit.

▶▶▶

DNA Isolation Kit for Cells and Tissues

The kit offers a convenient, rapid method for the large scale isolation of intact, high molecular weight, genomic DNA from tissues, cultured cells, *E. coli*, yeast, and mouse tails.

The amount of genomic DNA recovered with the DNA Isolation Kit for Cells and Tissues is significantly higher than that obtained using alternative column-based methods in significantly less time. Starting material quantities ranging from 100 mg to 1 gram of tissue, or from 1 x 10^7 to 5 x 10^7 cultured cells, can be analyzed with the kit. Within 2 h plus resuspension time (for tissue samples), the kit yields pure genomic DNA suitable for amplification of long fragments by standard PCR, restriction enzyme digestion, and Southern blotting.

TriPure Isolation Reagent

Analysis of gene expression requires clean, intact RNA templates. Isolation of intact RNA is complicated primarily by a host of stable cellular ribonucleases. To minimize RNase activity, RNA isolation procedures typically begin with cell or tissue lysis in a strongly denaturing environment. The RNA is then separated from other cellular components via multiple phenol/chloroform extractions or time-consuming CsCl step gradients.

A simpler alternative, the TriPure Isolation Reagent, offers a rapid RNA isolation procedure that can easily process multiple samples and produces 30 – 150% more RNA than other purification methods. Briefly, the reagent causes RNA, DNA, and protein separate into different organic phases, from which each can be purified by a series of alcohol precipitations (Chomczynski, 1993).

Thus, the TriPure Isolation Reagent allows the simultaneous isolation of RNA, DNA, and protein from the same sample. The isolated total RNA is suitable for Northern blots, RT-PCR, poly(A$^+$) fractionation, *in vitro* translation, or RNase protection assays. The isolated RNA-free DNA may be used for PCR, restriction digest, or Southern blots. The denatured protein may be analyzed on a Western blot.

References

Chomczynski, P. (1993) *BioTechniques* **15**, 532 – 537

Erlich, H.A., ed. (1989) *PCR Technology: Principles and Applications for DNA Amplification*, p. 31 – 38, Stockton Press, New York

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, vol. 2, p. 9.14 – 9.19, Cold Spring Harbor (N.Y.) Laboratory Press

DNA Isolation Kit for Mammalian Blood

for isolation of genomic DNA from 25 samples (10 ml each)

Cat. No. 11 667 327 001

Principle	The kit procedure starts with preferential lysis of erythrocytes. The remaining leukocytes are lysed with a strong anionic detergent, and proteins are removed by dehydration and precipitation. The purified genomic DNA is then recovered via ethanol precipitation.
Starting material	• 1 – 10 ml whole blood (research samples) that has been treated with an anticoagulant (sodium heparin, sodium citrate, or EDTA) *The kit works best with fresh blood or blood stored (at +2 to +8°C or –15 to –25°C) for ≤3 days. It should not be used with blood that is more than 1 month old or blood that has been frozen and thawed more than 3 times.* • Lymphocyte preparations from 10 ml mammalian blood (research samples) • Buffy coat preparations from 10 – 20 ml mammalian blood (research samples)
Application	• For preparation of high molecular weight, purified genomic DNA, which may be used in standard and long-template PCR, Southern blots, etc. *The kit has been used to prepare DNA from human, mouse, rat, dog, porcine, guinea pig, monkey, rabbit, and bovine blood.*
Time required	• Total time: approx. 90 min (plus 10 – 30 min to resuspend DNA) • Hands-on time: <50 min
Results	Yield: Variable, depending on species (see the table under Part IV of "How to use the kit" in this article). Purity: Average A_{260}/A_{280} of isolated DNA = 1.7 – 1.9.
Benefits	• **Saves time,** because the kit can prepare DNA directly from whole blood without prior isolation of leukocytes or buffy coat. • **Increases lab efficiency**, because the kit can prepare multiple DNA samples, free of RNA and protein, in approx. 90 min. • **Increases lab safety**, because the kit does not require extensive handling of potentially hazardous samples, nor use hazardous organic reagents. • **Accommodates "real world" samples with varying amounts of leukocytes**, because the sample volume can be adjusted from 1 – 10 ml.

- If you want to collect the DNA by spooling, then:
  - Wind the DNA strands around a sterile, blunt-ended glass rod.
  - Dip the glass rod containing the DNA into 3 ml cold 70% ethanol in a new sterile 50 ml centrifuge tube.
  - Swirl the glass rod until the DNA strands are released into the 70% ethanol.
  - Go to Step 14.

14. Centrifuge the tube containing 70% ethanol and DNA for 5 min at 875 x *g*. Discard the supernatant.

15. Dry the DNA pellet by:
    - EITHER placing the tube under vacuum **without heat** until the ethanol is no longer visible (usually <5 min).
    - OR inverting the tube and allowing the sample to air dry.

16. To rehydrate and dissolve the DNA pellet:
    - Add 1 ml of Tris-EDTA (pH 8.0) (other buffer) to the tube.
    - Vortex thoroughly.
    - Incubate at +65°C for 30 – 60 min.

      For DNA from human blood, use a 30 min incubation. For DNA from other mammalian species, use a 60 min incubation.
    - During the incubation, periodically vortex the tubes to facilitate solubilization of the DNA.

      Do not incubate the tube for >60 min at +65°C.
    - Store DNA at +2 to +8°C.

VI. Troubleshooting the DNA Isolation protocol

If you get...	Then, the cause may be...	And you should...
A cloudy upper layer and a red lower layer at Step 4	Little or no red cell lysis	▶ Start with fresh samples and repeat Steps 1 – 4 of the protocol with one or more changes: ▶ Be sure blood is at +15 to +25°C before starting protocol. ▶ Use a 15 min incubation in Step 3. ▶ If mixing by hand in Step 3, invert the tube more frequently.
Particles present in sample after vortexing (Step 7)	Incomplete white cell lysis	Using fresh samples, repeat Protocol Va, with one or more changes: ▶ In Step 6, be sure the white cell pellet is fully resuspended in the residual supernatant after vortexing. ▶ After adding buffer in Step 7, vortex the sample until the solution is dark brown/red and contains no particles. ▶ In Step 7, add enough White Blood Cell Lysis Buffer to ensure the solution is not viscous and does not contain clumps of cells. ▶ In Step 7, incubate the tube at +37°C for 15 – 30 min to facilitate lysis.

Typical results with the kit

Experiment 1

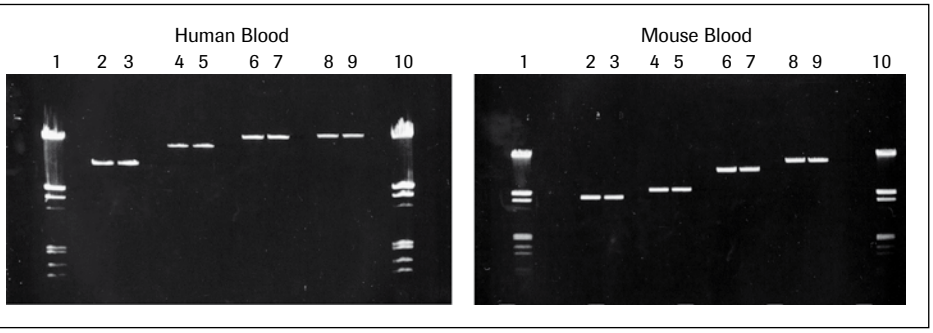


Figure 46: Use of DNA from human and mouse blood (prepared with the DNA Isolation Kit for Mammalian Blood) as templates for the amplification of large DNA fragments. Human and mouse blood research samples were collected as previously described (Noeth and Dasovich-Moody, 1997). DNA was prepared from 10 ml of each sample according to the protocol. Aliquots of each DNA preparation were used as templates for the long-template PCR amplification of several gene fragments. Each amplification procedure was performed with the Expand Long Template PCR System, using instructions given in the package insert.

Left panel shows gene fragments amplified from human DNA:
Lanes 1, 10: DNA Molecular Weight Marker III
Lanes 2, 3: tPA fragment (9.3 kb) amplified from 25 ng DNA
Lanes 4, 5: tPA fragment (15 kb) amplified from 50 ng DNA
Lanes 6, 7: β-globin fragment (23 kb) amplified from 100 ng DNA
Lanes 8, 9: β-globin fragment (28 kb) amplified from 200 ng DNA

Right panel shows the following fragments amplified from mouse DNA:
Lanes 1, 10: DNA Molecular Weight Marker III
Lanes 2, 3: IL-2 gene (4.2 kb) amplified from 330 ng DNA
Lanes 4, 5: α-2 collagen fragment (5.6 kb) amplified from 100 ng DNA
Lanes 6, 7: α-2 collagen fragment (10.4 kb) amplified from 50 ng DNA
Lanes 8, 9: α-2 collagen fragment (15.4 kb) amplified from 100 ng DNA

Result: All fragments were clearly visible on gels.

Experiment 2

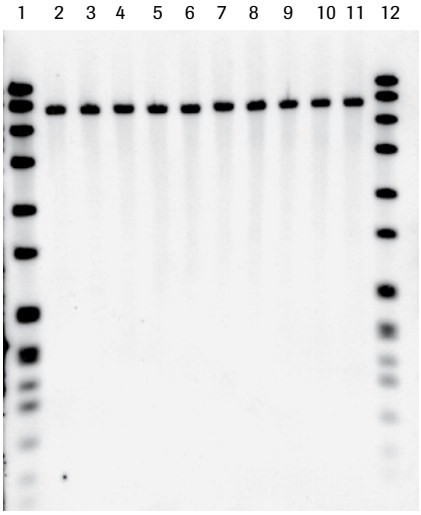
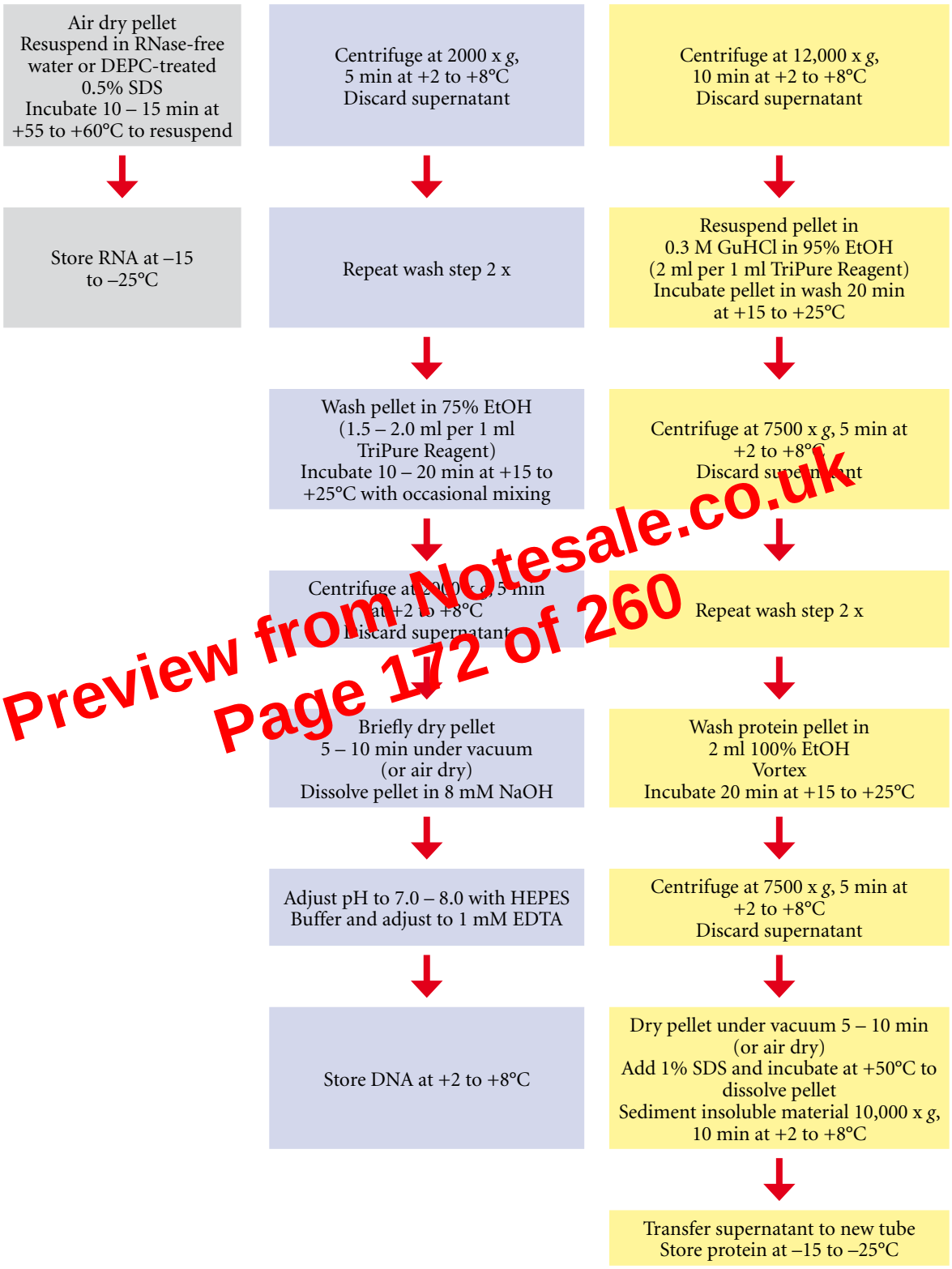


Figure 47: Use of DNA from various human blood research samples (prepared with the DNA Isolation Kit for Mammalian Blood) for detection of the n-ras gene by Southern hybridization. The above protocol was used to prepare DNA from several human blood samples, each of which had been prepared with a different anticoagulant. DNA was also prepared from a lymphocyte preparation and a buffy coat preparation, using a modification of the protocol outlined in the kit package insert. 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. 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

Result: Each lane contained only a single hybridization band of the expected (7.2 kb) size (Taparowsky et al., 1983).



mRNA Isolation Kit

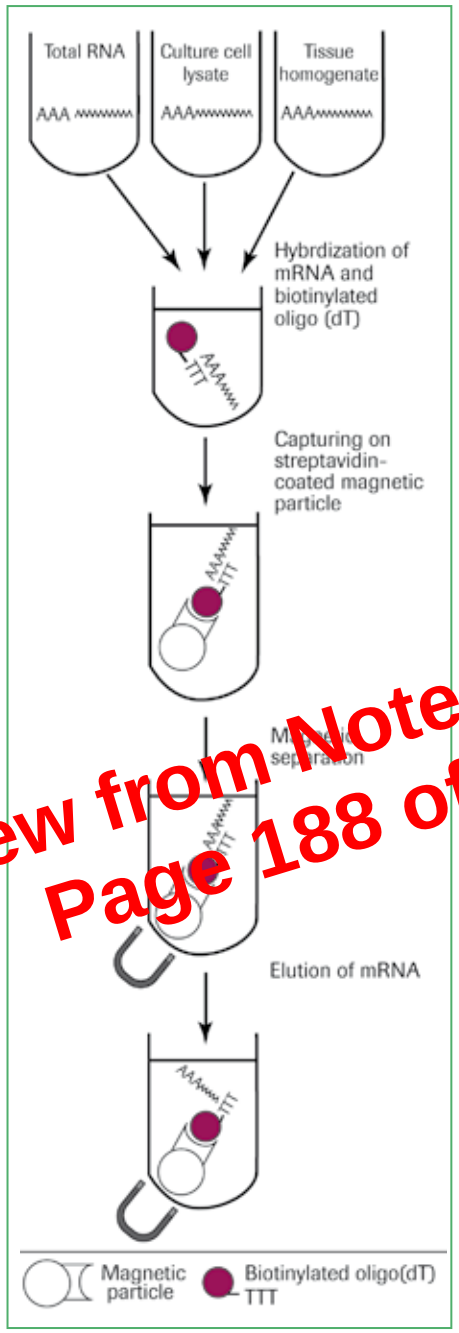
for the isolation of poly(A)$^+$ RNA (at least 70 µg)

Cat. No. 11 741 985 001

Principle	The poly(A)$^+$ tail of mRNA hybridizes to a biotin-labeled oligo(dT) probe. Streptavidin-coated magnetic particles capture the biotinylated dT-A hybrids. A magnetic particle separator collects the magnetic particles, and washes remove contaminants. Then, water elutes the mRNA from the particles.
Starting material	Research samples may contain up to: • 2.5 mg total RNA • 10^8 cultured cells • 1 g tissue
Application	Preparation of highly purified poly(A)$^+$ RNA, which may be used for RT-PCR, cDNA synthesis, Northern blotting, Northern ELISA, RNase protection assays, *in vitro* translation, etc.
Time required	• Total time: approx. 30 min (starting from total RNA) • Hands-on time: approx. 15 min (starting from total RNA)
Results	• Yield: Variable, depending upon starting material (See Part IV of "How to use the kit" in this article). • Purity: Isolated mRNA, free of DNA and other RNAs
Benefits	• **Saves time**, because the kit can isolate mRNA directly from cell lysates and tissue homogenates; no isolation of total RNA required • **Accommodates a wide range of samples**, and allows both small and large scale preparations of mRNA • **Increases lab safety**, because the kit does not use hazardous organic solvents.

How to use the kit

I. Flow diagram



- Cultured cells: Wash cells (2 x 10^7) twice with ice-cold phosphate-buffered saline (PBS). Add 3 ml Lysis Buffer to the cell pellet. Shear DNA mechanically by passing the sample 6 x through a 21-gauge needle.
- Tissue: Snap freeze 200 mg of tissue. Grind frozen tissue to a homogeneous powder in a pre-cooled mortar. Chill 3 ml Lysis Buffer to ≤0°C in a sodium chloride-ice water bath, then add the frozen powder to the chilled Lysis Buffer. Homogenize the powder suspension by passing it 4 x through a 21-gauge needle. Centrifuge the suspension at 11,000 x *g* for 30 s. Use only the supernatant for the capture procedure.

Perform all steps in the capture procedure below at a temperature between 0°C and –4°C.

2. Prepare the Streptavidin-coated Magnetic Particles:
   - Resuspend Streptavidin-coated Magnetic Particles thoroughly and pipette 300 µl Streptavidin-coated Magnetic Particles into a fresh cup or tube.
   - Immobilize the Streptavidin-coated Magnetic Particles on the side of the container with a Magnetic Particle Separator.
   - Remove the storage buffer from the Streptavidin-coated Magnetic Particles.
   - Resuspend the Streptavidin-coated Magnetic Particles in 500 µl Lysis Buffer.
   - Again immobilize the Streptavidin-coated Magnetic Particles with the magnetic separator and remove all the Lysis Buffer.

   Never let the Streptavidin-coated Magnetic Particles dry out.
3. Hybridize the mRNA to the Biotin-labeled Oligo(dT)$_{20}$:
   - Add 3 µl Biotin-labeled Oligo(dT)$_{20}$ to the sample (from Step 1).
   - Mix sample and Oligo(dT)$_{20}$ well to form the hybridization mix.
4. Immobilize the biotinylated dT-A hybrids with Streptavidin-coated Magnetic Particles:
   - Add hybridization mix to the tube containing the prepared Streptavidin-coated Magnetic Particles (from Step 2).
   - Resuspend Streptavidin-coated Magnetic Particles in the hybridization mix.
   - Incubate for:

     EITHER 5 min at +37°C (if you started with total RNA or cultured cells).

     OR 5 min at 0°C (if you started with tissue).
5. Separate the Streptavidin-coated Magnetic Particles from the fluid with a Magnetic Particle Separator.

   This step takes about 3 min.
6. Wash the Streptavidin-coated Magnetic Particles 3 times. For each wash:
   - Add 500 µl Wash Buffer to the tube.
   - Resuspend the Streptavidin-coated Magnetic Particles in the Wash Buffer.
   - Separate the Streptavidin-coated Magnetic Particles from the buffer with a Magnetic Particle Separator.
   - Remove all Wash Buffer and discard.
7. Elute the mRNA:
   - Add 50 µl redistilled water to the Streptavidin-coated Magnetic Particles.
   - Resuspend the Streptavidin-coated Magnetic Particles in the redistilled water.
   - Incubate for 2 min at +65°C.
   - Separate Streptavidin-coated Magnetic Particles from the eluate with a Magnetic Particle Separator.
8. Transfer the supernatant (containing the mRNA) to a fresh RNase-free tube.

   To quantitate the mRNA spectrophotometrically, assume that 1 A_{260} unit corresponds to a concentration of 40 µg/ml.

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Gel Filtration

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IV. Protocol for purifying radiolabeled nucleic acids

1. Remove the column from the storage bag, and gently invert it several times to resuspend the matrix.
2. Remove most of the buffer from the column as follows:
   - ▶ Remove the top cap from the column, then remove the bottom tip.
   - ▶ Drain the column by gravity, then discard the eluate.
3. Remove residual buffer from the column as follows:
   - ▶ Place the column in a collection tube.
   - ▶ Centrifuge at 1100 x *g* for 2 min in a swinging-bucket rotor.
   - ▶ Discard the collection tube with the eluted buffer.
4. While keeping the column upright, very carefully apply the sample to the center of the column bed.
   - ! *Apply 20 – 50 µl sample to a G-25 column; 20 – 100 µl to a G-50 column.*
5. While keeping the column upright, place the column in the second collection tube.
6. Centrifuge the tube at 1100 x *g* for 4 min in a swinging-bucket rotor.
7. Save the eluate from the second collection tube. It contains the purified sample.
8. Discard the column in a radioactive waste container.

V. Troubleshooting the Quick Spin protocol

If you get...	Then, the cause may be...	And you should...
Poor sample recovery	Tipping of the column, which causes back flow of sample and reduced nucleic acids recovery	▶ Keep the column upright during and after application of sample, especially after centrifugation.
	Wrong g-force used during centrifugation	▶ Use 1100 x *g* for centrifugation spins. ▶ Be sure the centrifuge is correctly calibrated.
	Wrong rotor	▶ Use swinging-bucket rotor. ! *A fixed-angle rotor will cause lower yields.*
	Too much or too little DNA/RNA loaded on column	▶ Apply a sample containing 0.02 – 1.0 mg/ml nucleic acid. At >1.0 mg/ml, the sample is viscous and may not migrate through the columns easily, leading to poor recovery and/or contamination with smaller molecules. At <0.02 mg/ml, DNA/RNA recovery may be low. ▶ To improve recovery of dilute samples, add carrier (glycogen, tRNA or sperm DNA) to the sample.

▶▶▶

Typical result with the columns

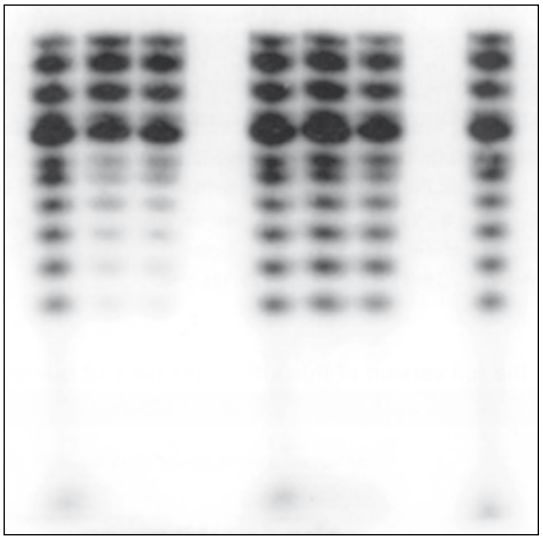

Figure 58: Removal of Unincorporated Radiolabeled Nucleotides from 5' End-labeled Oligomers using the Mini Quick Spin DNA and Oligo Columns. Various oligomers, ranging in size from 32 bp to 8 bp, were end-labeled using the 5' End-Labeling Kit and γ^{32}P-ATP and purified using either the mini Quick Spin DNA or Oligo Columns to remove unincorporated nucleotide, per manufacturer's instructions. One fifth of the isolated sample was electrophoresed through a 20% acrylamide gel in 1x TBE for 5 – 6 hours at 150 V. The gel was overlaid with plastic wrap and exposed to Lumi-Film for approximately 18 h.

Lane 1: Oligo Marker Control
Lane 2: Oligo Marker/miniQuick Spin DNA Column
Lane 3: Oligo Marker/mini Quick Spin DNA Column
Lane 4: –
Lane 5: Oligo Marker Control
Lane 6: Oligo Marker/mini Quick Spin Oligo Column
Lane 7: Oligo Marker/mini Quick Spin Oligo Column
Lane 8: –
Lane 9: Oligo Marker Control

Results: Removal of Unincorporated Radio-labeled Nucleotides from 5' End-labeled Oligomers using the Mini Quick Spin DNA and Mini Quick Spin Oligo Columns

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Ordering Guide

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Product	Application	Cat. No.	Pack Size
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MagNA Lyser Rotor Cooling Block#	Houses the MagNA Lyser Rotor to maintain the temperature of the samples at +2 to +8°C	03 359 085 001	1 cooling block
MagNA Lyser Green Beads#	Specially designed ceramic beads to achieve optimal homogenization of various sample materials	03 358 941 001	100 tubes (prefilled with ceramic beads)

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