


# High Pure RNA Isolation Kit

for isolation of total RNA from up to 50 samples

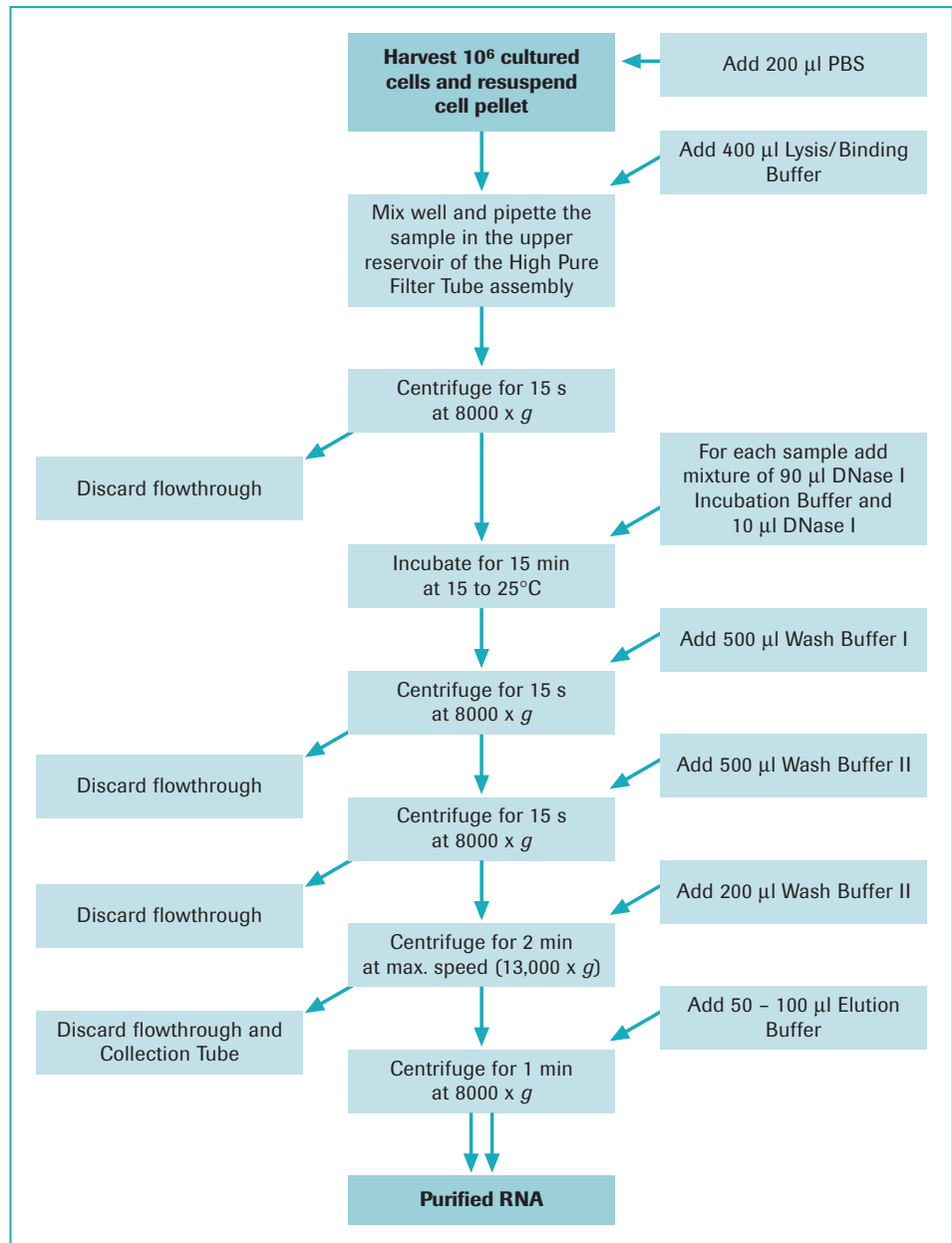
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|--------------------------|---|
| <b>Principle</b>         | A single reagent lyses the sample and inactivates RNase. In the presence of a chaotropic salt (guanidine HCl), the released total RNA binds selectively to glass fiber fleece in a special 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. |
| <b>Starting material</b> | <ul style="list-style-type: none"> <li>● 10<sup>6</sup> cultured mammalian cells (research samples)</li> <li>● 200 – 500 µl human whole blood (research samples), containing anticoagulant</li> <li>● 10<sup>8</sup> yeast (mid- to late-log phase, A<sub>600</sub> &lt;2.0)</li> <li>● 10<sup>9</sup> gram positive or gram negative bacteria</li> </ul>   |
| <b>Application</b>       | <ul style="list-style-type: none"> <li>● 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, <i>in vitro</i> translation, or nuclease protection assays</li> </ul> <p> RNA prepared from human whole blood is suitable for RT-PCR only.</p>                             |
| <b>Time required</b>     | <ul style="list-style-type: none"> <li>● Total time: approx. 25 min (+ sample pretreatment, if needed)</li> <li>● Hands-on time: &lt;10 min (+ sample pretreatment, if needed)</li> </ul>   |
| <b>Results</b>           | <ul style="list-style-type: none"> <li>● Yield: Variable, depending on sample type (See the table under Part IV of “How to use the kit” in this article)</li> <li>● Purity: Purified RNA is free of DNA, protein, salts, and other cellular components</li> </ul>   |
| <b>Benefits</b>          | <ul style="list-style-type: none"> <li>● <b>Saves time</b>, because the kit can produce multiple RNA samples in minutes</li> <li>● <b>Ideal for a wide variety of samples</b>, because one kit can purify RNA from many sources</li> <li>● <b>Minimizes RNA loss</b>, because the kit removes contaminants without time-consuming precipitation or solvent extraction</li> <li>● <b>Increases lab safety</b>, because the kit does not use hazardous organic solvents</li> </ul>  |

## How to use the kit

### I. Flow diagram



### II. Kit contents

- Lysis/Binding Buffer containing guanidine HCl and Triton X-100 (25 ml)
- DNase I, lyophilized (10 kU)
  - ! Dissolve DNase I in 0.55 ml Elution Buffer and store in aliquots at  $-15$  to  $-25^\circ\text{C}$ . A 0.11 ml aliquot is enough to process 10 samples.
- DNase Incubation Buffer (10 ml)
- Wash Buffer I containing guanidine HCl (33 ml)
  - ! Add 20 ml absolute ethanol to Wash Buffer I before use.

- Wash Buffer II (10 ml)
  - ! Add 40 ml absolute ethanol to Wash Buffer II before use.
- Elution Buffer (30 ml)
- High Pure Filter Tubes (50 tubes)
- Collection Tubes, 2 ml (50 tubes)

### III. Additional materials needed

- Absolute ethanol
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- Phosphate buffered saline (PBS), sterile
- Red Blood Cell Lysis Buffer (for human blood)
- Lysozyme, 50 mg/ml (for bacteria)
- 10 mM Tris-HCl, pH 8.0, sterile (for bacteria)
- Lyticase, 0.5 mg/ml (for yeast)
- Standard tabletop centrifuge capable of 3000 x g centrifugal force (for yeast or bacteria)

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

| Starting material               | Sample size           | Average RNA yield (µg) |
|---------------------------------|-----------------------|------------------------|
| Cultured cells (K 562)          | 10 <sup>6</sup> cells | 15                     |
| Whole blood, human              | 200 – 500 µl          | 10 RT-PCR              |
| Yeast ( <i>S. cerevisiae</i> )  | 10 <sup>8</sup> cells | 20                     |
| Bacteria ( <i>E. coli</i> )     | 10 <sup>9</sup> cells | 50                     |
| Bacteria ( <i>B. subtilis</i> ) | 10 <sup>9</sup> cells | 35                     |

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## V. Protocols for preparing total RNA



*Be careful to avoid RNase contamination throughout these protocols. For details, see the package insert provided with the kit.*

### Va. Isolation of total RNA from 10<sup>6</sup> cultured mammalian cells



*This protocol may also be used to isolate RNA from small blood volumes (<200 µl), as an alternative to Protocol Vb.*

- 1 Harvest about 10<sup>6</sup> cultured mammalian cells and resuspend in 200 µl PBS.
- 2 Add 400 µl Lysis/Binding Buffer to the resuspended cells. Mix the contents of the tube well.
- 3 To transfer the sample to a High Pure Tube:
  - ▶ Insert one High Pure Filter Tube into one Collection Tube.
  - ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube (max. 700 µl).
- 4 Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge, then:
  - ▶ Centrifuge the tube assembly for 15 s at approx. 8000 x *g*.
  - ▶ Separate the two tubes and discard the liquid in the Collection Tube.
  - ▶ Reinsert the Filter Tube in the same Collection Tube.
- 5 In a separate, sterile tube, mix 100 µl DNase solution (90 µl DNase Incubation Buffer + 10 µl reconstituted DNase I) for each sample. Then:
  - ▶ Add 100 µl DNase solution to the upper reservoir of the Filter Tube.
  - ▶ Incubate the Filter Tube for 15 min at 15 to 25°C.
- 6 After the DNase incubation:
  - ▶ Add 500 µl Wash Buffer I to the upper reservoir of the Filter Tube.
  - ▶ Repeat the centrifugation (as in Step 4).
- 7 After the first wash:
  - ▶ Add 500 µl Wash Buffer II to the upper reservoir of the Filter Tube.
  - ▶ Repeat the centrifugation (as in Step 4).
- 8 After the second wash:
  - ▶ Add 200 µl Wash Buffer II to the upper reservoir of the Filter Tube.
  - ▶ Centrifuge the tube assembly for 2 min at maximum speed (approx. 13,000 x *g*) to remove any residual Wash Buffer.
- 9 Discard the Collection Tube and insert the Filter Tube in a clean, sterile 1.5 ml microcentrifuge tube.
- 10 To elute the RNA:
  - ▶ Add 50 – 100 µl Elution Buffer to the Filter Tube.
  - ▶ Centrifuge the tube assembly for 1 min at 8000 x *g*.






*The microcentrifuge tube now contains the eluted total RNA, which may be used directly in a variety of procedures.*


## Vb. Isolation of total RNA from 500 µl human blood (research samples)

### General notes:

- ▶ The blood should be fresh and at 15 to 25°C. It should have been collected in a tube containing sodium EDTA or any other anticoagulant.
- ▶ This protocol may be used to prepare RNA from 200 – 500 µl blood.

- 1 Prepare sample tubes:
  - ▶ Warm Red Blood Cell Lysis Buffer to 15 to 25°C.
  - ▶ For each sample, add 1 ml Red Blood Cell Lysis Buffer to a sterile 1.5 ml microcentrifuge tube.
- 2 To each microcentrifuge tube:
  - ▶ Add 500 µl human whole blood.
  - ▶ Cap and mix by inversion.
  - ▶  *Do not vortex tubes.*
- 3 Mix the tubes by:
  - ▶ EITHER placing the microcentrifuge tube on a rocking platform or gyratory shaker for 10 min.
  - ▶ OR inverting the sample at regular intervals by hand, for a total of 10 min.
- 4 Centrifuge the tube at 500 x *g* for 5 min in a microcentrifuge.
- 5 With a sterile pipette, carefully remove and discard the clear, red supernatant.
- 6 For each tube:
  - ▶ Add 1 ml Red Blood Cell Lysis Buffer to the white pellet, then cap the tube.
  - ▶ “Flick” the tube (with fingers) until the pellet is completely resuspended.
  - ▶  *Do not vortex the tube.*
- 7 Centrifuge the tube at 500 x *g* for 3 min.
- 8 With a sterile pipette, carefully remove and discard both the supernatant and the red ring of blood cell debris that forms around the outer surface of the white blood cell pellet.
- 9 The white blood cell pellet is the starting material for RNA isolation. Thus:
  - ▶ Resuspend the white pellet in 200 µl sterile PBS.
- 5 Follow Protocol Va above, starting with the addition of Lysis/Binding Buffer (Step 2).

 *Alternatively blood stabilized with the RNA/DNA Stabilization Reagent for Blood/ Bone Marrow can be used*

- 1 Mix 0.5 ml whole blood with 1 ml reagent.
  - ▶ This mixture is stable for at least one week at 2 to 8°C or a month at -15 to -25°C.
- 2 900 µl lysate (corresponding to 300 µl blood) is transferred to the Filter Tube and centrifuged for 1 min at 8000 x *g*
  - ▶  *Remove any residual lysis reagent by a second centrifugation step.*

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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  $2000 \times g$  for 5 min in a standard tabletop centrifuge.
  - ▶ Resuspend the pellet in 200  $\mu$ l of PBS and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.
- 2 Add 10  $\mu$ l lyticase solution (0.5 mg/ml) to each microcentrifuge tube. Incubate the tube for 15 min at  $30^\circ\text{C}$ .
- 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)

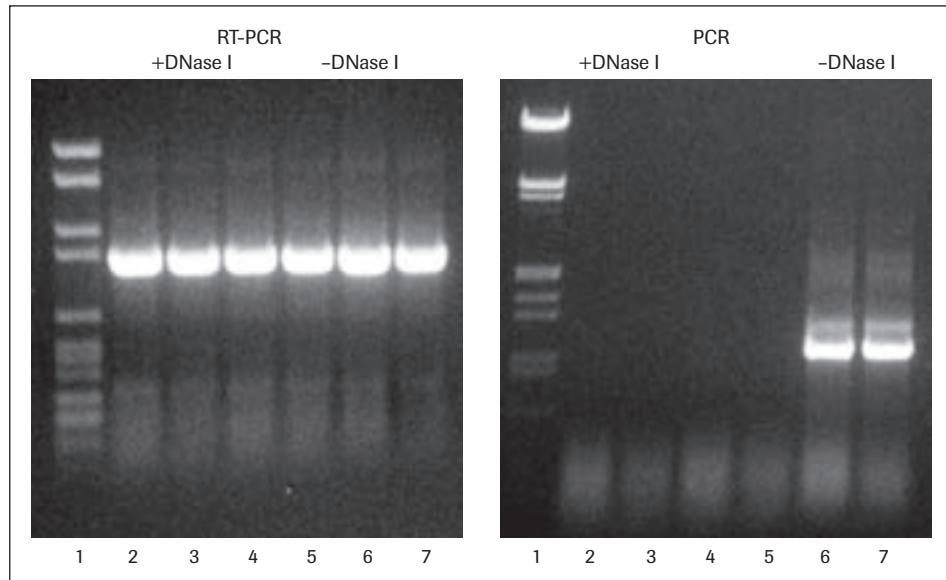
- 1 For each sample ( $10^9$  bacteria):
  - ▶ Collect the bacteria by centrifugation at approximately  $2000 \times g$  for 5 min in a standard tabletop centrifuge.
  - ▶ Resuspend the pellet in 200  $\mu$ l of 10 mM Tris-HCl, pH 8.0, and transfer the suspension to a sterile 1.5 ml microcentrifuge tube.
- 2 Add 4  $\mu$ l lysozyme solution (50 mg/ml in Tris-HCl, pH 8.0) to each microcentrifuge tube. Incubate the tube for 10 min at  $37^\circ\text{C}$ .
- 3 Add 400  $\mu$ l Lysis/Binding Buffer to the tube and mix well.
- 4 To transfer the sample to a High Pure Tube:
  - ▶ Insert one High Pure Filter Tube into one Collection Tube.
  - ▶ Pipette entire sample into upper buffer reservoir of the Filter Tube.
- 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  $\mu$ l DNase solution to the upper reservoir of the Filter Tube.
  - ▶ Incubate the Filter Tube for 60 min at 15 to  $25^\circ\text{C}$ .
- 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 98 of this manual. For factors that may affect the High Pure RNA Isolation Kit, see page 102.

## Typical results with the kit

### Experiment 1



**Figure 12: Effect of DNase treatment on mammalian RT-PCR templates prepared with the High Pure RNA Isolation Kit.** RNA was isolated from six identical samples (each containing  $10^6$  K 562 human lymphocyte cells). Four samples were treated with the DNase solution as described in Protocol Va above. For 2 samples, the DNase step was omitted. 10  $\mu$ l of each sample was used in a first strand cDNA reaction (20  $\mu$ l total volume) with M-MuLV reverse transcriptase and oligo(dT) (as primer). From this first strand cDNA reaction, 10  $\mu$ l were transferred to a PCR (Expand High Fidelity system) and amplified with a primer pair specific for the GAPDH gene.

**Left panel:**

**Lane 1:** MWM VI

**Lanes 2 – 7:** The RT-PCR produced a specific 983 bp amplification product.

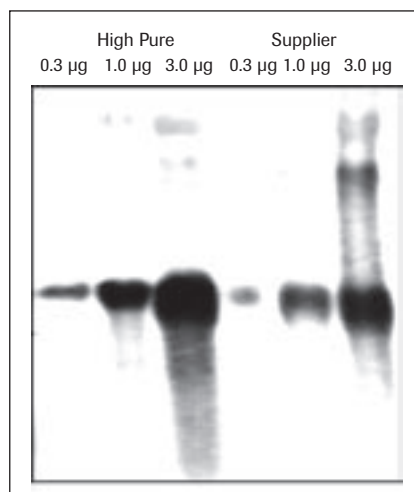
**Right panel:**

**Lane 1:** MWM III

**Lanes 2 – 7:** In a control PCR, the samples were processed as for RT-PCR, except the reverse transcriptase in the first strand cDNA reaction was omitted.

**Result:** The samples not incubated with DNase gave an amplification product in the PCR control, indicating the presence of some residual genomic DNA (right panel, lanes 6 and 7), samples that were treated with DNase did not give any amplification product (right panel, lanes 2 – 5) in the control.

### Experiment 2



**Figure 13: Northern analysis of RNA prepared with the High Pure RNA Isolation Kit and a competitor's kit.** RNA was isolated from  $10^6$  K 562 human lymphocyte cells with each kit, and the yields determined spectrophotometrically. The indicated amount of each RNA sample was loaded on a denaturing formaldehyde gel, separated electrophoretically, and transferred to a positively charged nylon membrane (Roche Applied Science) by capillary blotting. The blot was hybridized with a digoxigenin-labeled actin RNA probe (Roche Applied Science). Hybridized probe was detected immunochemically and visualized with CSPD chemiluminescence substrate.

**Result:** A specific signal of the expected size was obtained in all samples, but the specific actin RNA bands in the aliquots prepared with the Roche Applied Science kit were more concentrated and contained less background.

### Experiment 3

Analysis with the LightCycler® Instrument of cDNA synthesis derived from total RNA isolated according to the alternative blood protocol forming a lysate with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow (HP-VB) and of a white blood cell lysate (HP-WBC).

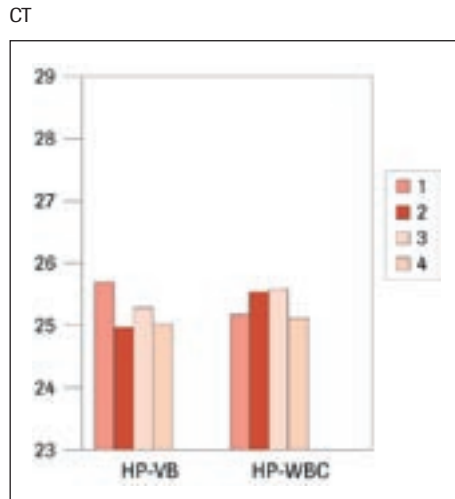


Figure 14: Mammaglobin

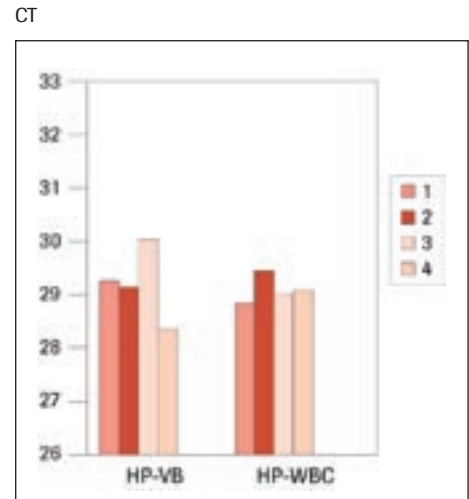
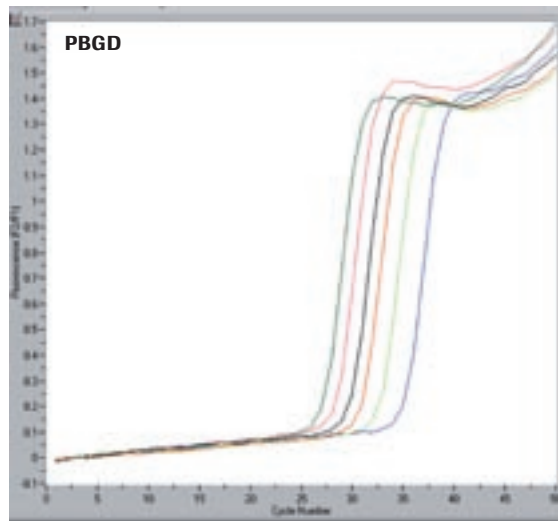


Figure 15: PBGD



**Figure 16: High Pure total RNA Preparation from human whole blood (research samples).** The porphobilinogen deaminase (PBGD) mRNA reaction product serves as control for RT-PCR performance and as reference for relative quantification.

- 10 µl Blood = 30 µl Lysate
- 20 µl Blood = 60 µl Lysate
- 50 µl Blood = 150 µl Lysate
- 100 µl Blood = 300 µl Lysate
- 200 µl Blood = 600 µl Lysate
- 300 µl Blood = 900 µl Lysate

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