High Pure RNA Isolation Kit

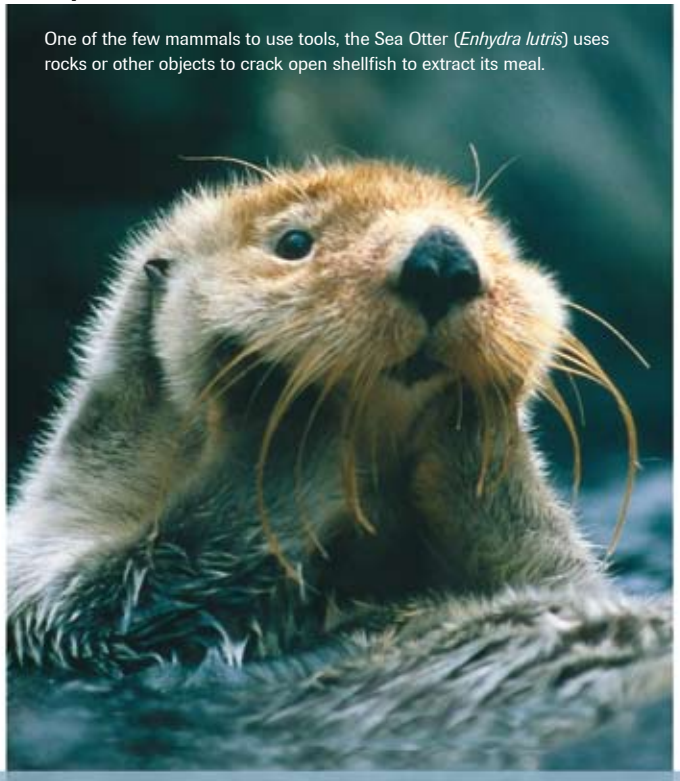
Simplify small-scale isolation of total RNA

Choose the **High Pure RNA Isolation Kit** to rapidly isolate intact total RNA from a broad range of research sample materials, including cultured cells, mammalian blood, white blood cells (WBCs), yeast, and bacteria.

Recover highly pure, concentrated RNA (in 50 µl) from one sample in 25 minutes, and process multiple samples in 45 minutes with a straightforward workflow.

Produce high-quality template for direct use in cDNA library construction, RT-PCR, qRT-PCR, northern blotting, differential display, nuclease protection assays, primer extension, RACE, and *in vitro* translation.

Experts at Extraction



One of the few mammals to use tools, the Sea Otter (*Enhydra lutris*) uses rocks or other objects to crack open shellfish to extract its meal.

■ Efficiently isolate RNA from diverse sample types with one versatile kit.

Conserve resources by using a single kit for multiple applications - eliminating the need to use several kits from other suppliers.

■ Avoid genomic DNA contamination.

Rely on the kit's integrated on-column DNase-treatment step to degrade genomic DNA.

■ Generate high-purity template for optimal performance in downstream assays.

Obtain high sensitivity, reproducibility, and specificity in quantitative RT-PCR and other applications (Figure 1).

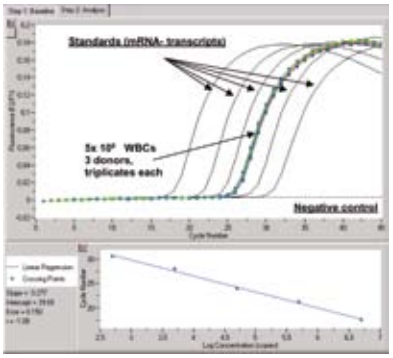


Figure 1: Sensitive quantification of low-copy RNA obtained with the High Pure RNA Isolation Kit. White blood cells from three different human research samples were prepared from EDTA-blood using Red Blood Cell Lysis Buffer. RNA from approximately 5 x 10^6 white blood cells of each sample (triplicates; colored lines with dots, squares, crosses) were isolated using the High Pure RNA Isolation Kit and analyzed in qRT-PCR using the LightCycler® h-HPRT Housekeeping Gene Set. The pre-diluted standard solutions of *in vitro*-transcribed hypoxanthine phosphoribosyl transferase (HPRT) RNA were used to generate a reference curve (black lines).
Result: The High Pure RNA Isolation Kit provides high template quality and sensitivity.

Efficiently purify total RNA

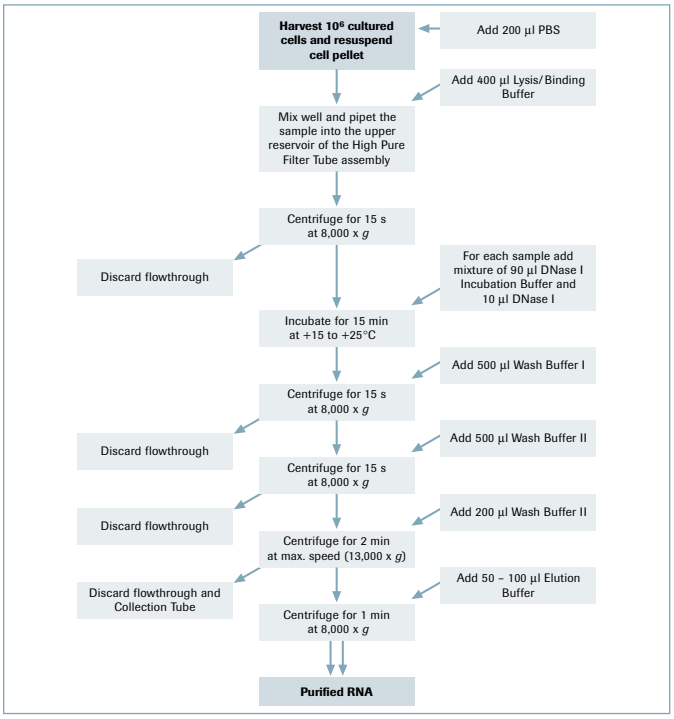
Procedure

Protocol for isolating total RNA from up to 10^6 cultured cells.

View detailed procedures for other sample materials in the pack insert at **www.roche-applied-science.com**

1. Resuspend cells in 200 µl PBS.
2. Add 400 µl Lysis/Binding Buffer and vortex for 15 s.
3. To transfer the sample to a High Pure Filter Tube:
   - Insert one High Pure Filter Tube into one Collection Tube.
   - Pipet the entire sample into the upper reservoir of the Filter Tube (max. 700 µl).
4. - Insert the entire High Pure Filter Tube assembly into a standard table-top centrifuge.
   - Centrifuge the tube assembly 15 s at 8,000 × *g*.
5. After centrifugation:
   - Remove the Filter Tube from the Collection Tube; discard the flowthrough, and again combine the Filter Tube and the used Collection Tube.
6. After re-inserting the Filter Tube:
   - For each sample, pipet 90 µl DNase I Incubation Buffer into a sterile reaction tube, add 10 µl DNase I, mix, and pipet the solution onto the glass fiber fleece in the upper reservoir of the Filter Tube.
   - Incubate for 15 min at +15 to +25°C.
7. - Add 500 µl Wash Buffer I to the upper reservoir of the Filter Tube assembly and centrifuge 15 s at 8,000 × *g*.
   - Discard the flowthrough and combine the Filter Tube with the used Collection Tube.
8. - Add 500 µl Wash Buffer II to the upper reservoir of the Filter Tube assembly and centrifuge 15 s at 8,000 × *g*.
   - Discard the flowthrough and combine the Filter Tube with the used Collection Tube.
9. Add 200 µl Wash Buffer II to the upper reservoir of the Filter Tube assembly and centrifuge for 2 min at maximum speed (approx. 13,000 × *g*) to remove any residual Wash Buffer.
   ! The extra centrifugation time ensures removal of residual Wash Buffer.
10. Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
11. To elute the RNA:
    - Add 50 – 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
    - Centrifuge the tube assembly for 1 min at 8,000 × *g*.
12. The microcentrifuge tube contains the eluted, purified RNA, which can be used directly in RT-PCR or stored at –80°C for later analysis.

High Pure RNA Isolation Kit workflow



Typical RNA yields from different sample materials

Starting Material	Sample Size	Average RNA Yield
Cultured cells (K-562)	10^6 cells	5 µg
Whole blood, human	200 µl	Enough for 10 RT-PCR reactions
Yeast *(S. cerevisiae)*	10^8 cells	20 µg
Bacteria *(E. coli)*	10^9 cells	50 µg
Bacteria *(B. subtilis)*	10^9 cells	35 µg

Figure 2: Typical yields obtained with the High Pure RNA Isolation Kit.

Ordering information

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

For more information about the **High Pure RNA Isolation Kit** and other products for nucleic acid isolation and purification, visit **www.roche-applied-science.com/napure**

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