

# RNAqueous<sup>®</sup> –4PCR Kit

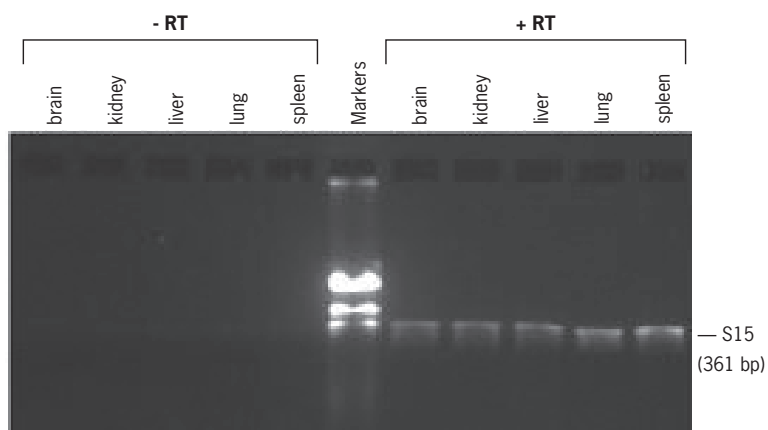
## DNA-free RNA Isolation for RT-PCR

- Safe – phenol free isolation and DNase removal
- Provides high quality total RNA for end point and real-time RT-PCR reactions
- Fast – obtain total RNA free of contaminating DNA in less than 45 minutes
- Excellent recovery of RNA – ideal for both cells and tissues
- Flexible – isolate total RNA from 1 to 75 mg of tissue or 100 to 10<sup>7</sup> cells

### RNAqueous Technology

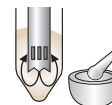
The RNAqueous-4PCR Kit provides RNA free of genomic DNA contamination from samples as small as 1 mg or 100 cells. The purified RNA is ready to use and is ideally suited for downstream applications that are sensitive to low amounts of DNA contamination, such as real-time or end point RT-PCR.

The DNase treatment and removal reagents consist of RNase-free DNase I, 10X DNase Buffer, and a DNase Removal Reagent. In addition, the kit contains three plastic pestles designed for disruption of small amounts of tissue.



**Figure 2. RT-PCR Experiments Using Total RNA Isolated with the RNAqueous™-4PCR Kit.** 5 µl of each RNA sample were used as templates for reverse transcription reactions and then 10 percent of the resulting cDNA was amplified by PCR using S15 primers. The lanes to the left of the markers are PCRs done without reverse transcription, demonstrating the lack of genomic DNA contamination in these RNA samples. The lanes to the right of the markers show the S15 RT-PCR product from the indicated samples.

Tissue disruption  
in Lysis Solution



Add EtOH,  
load on filter  
(0.5 min)  
in Lysis Solution



Centrifuge



Washes 1, 2 & 3  
(~5 min)



Elution  
(~10 min)



### Total RNA

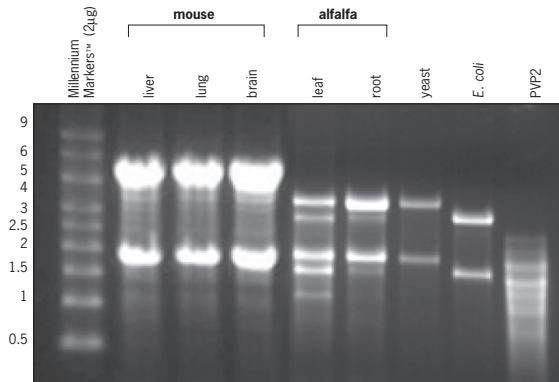
(Northern Analysis, Nuclease Protection, RT-PCR, Array Analysis, Translation)

\*RNAqueous<sup>®</sup>-Midi utilizes a filter adapted for a 10 ml syringe. Lysate and washes are pushed through the filter by the syringe plunger

**Figure 1. The RNAqueous Protocol.**

## Excellent Recovery of High Quality RNA

Yields of RNA will vary according to the type and amount of sample, but will typically fall in the range of 1–10 µg per mg tissue, or ~1 µg per 1 x 10<sup>5</sup> cells in culture.



**Figure 3. Total RNA From Several Different Tissues and Species Isolated Using RNAqueous®.** EtBr-staining of rRNA reveals RNA integrity. The gel is 1% agarose in formaldehyde/ MOPS buffer.

Also Available from Ambion	
RNAqueous®-Micro	The RNAqueous-Micro Kit is optimized for the purification of total RNA from micro sized samples such as 10-500,000 cultured cells, as few as 10 microdissected cells, or up to 10 mg tissue.
RNAqueous®-Midi	The RNAqueous-Midi Kit is designed for the purification of total RNA from 0.1 g to 0.5 g of tissue or 10 <sup>7</sup> -10 <sup>8</sup> cells. In this kit, a luer-lock syringe filter houses the RNA binding glass fiber filter.
RNAlater®	RNAlater is an aqueous, non-toxic tissue storage reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue samples. RNAlater eliminates the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing.
RNaseZap®	RNaseZap contains three ingredients active against RNase and has proven to be extremely effective at removing RNase contamination from glassware, plastic surfaces, countertops, and pipetters in our laboratories. It has also been shown to be effective at eliminating RNase contamination from microfuge tubes without inhibiting subsequent enzymatic reactions.

**Table 1. Total Yields Obtained with RNAqueous 4PCR Kit**

Sample	# of cells or mg of tissue	Average yield*
Jurkat cells	1 x 10 <sup>6</sup>	10 µg
	1 x 10 <sup>7</sup>	50 µg
<i>E. coli</i>	1 x 10 <sup>8</sup>	5 µg
	1 x 10 <sup>9</sup>	50 µg
Mouse liver	10 mg	40–60 µg
Mouse lung	10 mg	10–15 µg
Mouse spleen	10 mg	10–20 µg
Mouse kidney	10 mg	15–25 µg
Mouse brain	10 mg	8 µg

\*Amounts can vary due to factors such as species, developmental stage, and growth conditions. The total RNA yield does not include 5S rRNA, tRNA and other low-molecular weight RNAs, which can make up to 20% of total cellular RNA.

**Table 2. RNAqueous®-4PCR Spin Columns Specifications**

Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA > 200 nucleotides
Minimum elution volume	40 µl
Maximum amount of starting material	
Animal cells	1 x 10 <sup>7</sup> cells
Animal tissues	75 mg

## ORDERING INFORMATION

	CAT#	SIZE
RNAqueous®-4PCR Kit	1914	30 purifications

RELATED PRODUCTS	CAT#	SIZE
RNAqueous®-Midi	1911	15 purifications
RNAqueous®-Micro Kit	1931	50 purifications
RNAlater®	7020-7024	varies
RNaseZap®	9780	250 ml

For more information or to order online anytime, go to [www.ambion.com](http://www.ambion.com)



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