

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material to obtain optimal RNA yield and purity. The maximum amount that can be used is determined by:

- The type of sample and its RNA content
- The volume of Buffer RLT required for efficient lysis
- The RNA binding capacity of the RNeasy spin column

When processing samples containing high amounts of RNA, less than the maximum amount of starting material shown in Table 1 should be used, so that the RNA binding capacity of the RNeasy spin column is not exceeded.

When processing samples containing average or low amounts of RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the RNA binding capacity of the RNeasy spin column is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of RNA to the RNeasy spin column membrane, resulting in lower RNA yield and purity.

More information on using the correct amount of starting material is given in each protocol. Table 2 shows expected RNA yields from various sources.

Table 1. RNeasy Mini spin column specifications

Maximum binding capacity	100 µg RNA
Maximum loading volume	700 µl
RNA size distribution	RNA >200 nucleotides
Minimum elution volume	30 µl
Maximum amount of starting material	
■ Animal cells	1 × 10 ⁷ *
■ Animal tissues	30 mg*
■ Yeast	5 × 10 ⁷ *
■ Plant tissues	100 mg
■ Filamentous fungi	100 mg

* For larger sample sizes, RNeasy Kits and RNeasy Protect Kits are available in midi and maxi formats. For details, visit www.qiagen.com/RNA.

Note: If the binding capacity of the RNeasy spin column is exceeded, RNA yields will not be consistent and may be reduced. If lysis of the starting material is incomplete, RNA yields will be lower than expected, even if the binding capacity of the RNeasy spin column is not exceeded.

Table 2. Typical yields of total RNA with RNeasy Mini spin columns

Source	Yield of total RNA* (µg)
Cell cultures (1 x 10⁶ cells)	
■ NIH/3T3	10
■ HeLa	15
■ COS-7	35
■ LMH	12
■ Huh	15
Mouse/rat tissues (10 mg)	
■ Embryo (13 day)	25
■ Kidney	20–30
■ Liver	40–60
■ Spleen	30–40
■ Thymus	40–50
■ Lung	10–20
Yeast (1 x 10⁷ cells)	
■ <i>S. cerevisiae</i>	25
Plants (100 mg leaves)	
■ Arabidopsis	35
■ Maize	25
■ Tomato	65
■ Tobacco	60

* Amounts can vary due to factors such as species, developmental stage, and growth conditions. Since the RNeasy procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA, and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.