Quick-Start Protocol March 2016

RNAprotect® Bacteria Reagent and RNeasy® Protect Bacteria Kits

The RNAprotect Bacteria Reagent (cat. no. 76506) can be stored at room temperature (15–25°C) for up to 12 months if not otherwise stated on label. The RNeasy Mini Kit (cat. no. 74104) and the RNeasy Midi Kit (cat. no. 75142) can be stored at room temperature (15–25°C) for at least 9 months if not otherwise stated on label.

Further information

- *RNAprotect Bacteria Reagent Handbook*: **www.qiagen.com/HB-1704**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- For further protocols, including options for mechanical disruption of bacteria using the TissueRuptor® or TissueLyser instruments, please refer to the *RNAprotect Bacteria Reagent Handbook*. Protocols 1–6 provide instructions on stabilizing RNA and disrupting bacterial cells. The choice of protocol depends on the bacterial cell wall. Bacterial cells must be completely disrupted to ensure efficient RNA purification.
- If using RNeasy Kits for RNA purification, add 10 µl β-mercaptoethanol (β-ME) per 1 ml Buffer RLT, and mix. The mix is stable for 1 month.

1. Prepare TE buffer (30 mM Tris·Cl, 1 mM EDTA, pH 8.0) containing 15 mg/ml lysozyme.
2. Calculate the required volume of bacterial culture (1 volume). Pipet 2 volumes of RNAprotect Bacteria Reagent into a tube (not supplied).
3. Add 1 volume of bacterial culture to the tube. Mix immediately by vortexing for 5 s. Incubate for 5 min at room temperature (15–25°C).

4. Centrifuge for 10 min at 5000 x *g*.
5. Decant the supernatant. Remove residual supernatant by gently dabbing the inverted tube once onto a paper towel.
6. Add 10–20 µl QIAGEN Proteinase K to the appropriate volume of TE buffer containing lysozyme (see Table 1) and add the mixture to the pellet. Carefully resuspend the pellet by pipetting up and down several times.
7. Mix by vortexing for 10 s. Incubate at room temperature (15–25°C) for 10 min. During incubation, incubate on a shaker-incubator, or vortex for 10 s at least every 2 min.
8. Add the appropriate volume of Buffer RLT (see Table 1) and vortex vigorously. If particulate material is visible, pellet it by centrifugation, and use only the supernatant in step 9.
9. Add an appropriate volume of ethanol (see Table 1). Mix well by pipetting (RNeasy Mini procedure) or by shaking vigorously (RNeasy Midi procedure). Do not centrifuge.
10. If using RNeasy Mini Kit, proceed with step 3 in the *Quick-Start Protocol RNeasy Mini Kit, Part 1*. If using RNeasy Midi Kit, proceed with step 3 in the *Quick-Start Protocol RNeasy Midi Kit*.

Table 1. Reagent volumes for enzymatic lysis and Proteinase K digestion of bacteria

Number of bacteria	RNeasy spin column	TE with lysozyme (step 6)	Buffer RLT (step 8)	Ethanol (96–100%) (step 9)	Ethanol (80%) (step 9)
$<1 \times 10^8$	Mini	100 µl	350 µl	250 µl	–
1×10^8–2.5×10^8	Mini	200 µl	700 µl	500 µl	–
$<2.5 \times 10^8$–1.5×10^9	Midi	200 µl	2000 µl	–	1750 µl
7.5×10^8–1.5×10^9	Midi	200 µl	4000 µl	–	3500 µl

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