# *AccuPrep*® Bacterial RNA Extraction Kit (K-3142, K-3143)

## Ⅰ Before You Begin

1) Add 10 µl β-mercaptoethanol per 1 ml RB Buffer.
2) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
3) Prepare TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) containing **20 mg/ml lysozyme**.
4) Prepare 50 mg acid-washed glass beads (150-600 µm) per sample for RNA extraction from gram-positive bacteria.

## Ⅱ RNA Extraction from gram-negative bacteria

1) Calculate the volume of bacteria culture (1 volume).
2) Add **0.5 volume** of **RS Buffer** into a tube (not supplied).
3) Add **1 volume** of **bacteria culture** to the tube and mix by **vortex mixer** for **5 sec**. **Incubate** for **5 min** at room temperature (15~20˚C).
4) Centrifuge at **7,500 rpm** for **10 min**.
5) Discard the supernatant from the tube.
6) Add **20 µl** of **Proteinase K** to the 100 µl of TE buffer containing lysozyme, and add the mixture to the tube.
7) Resuspend the pellet by pipetting and mix by vortex mixer for 10 sec. **Incubate** for **10 min** at room temperature (15~20˚C).
8) Add **700 µl** of **RB Buffer** to the tube and mix by vortex mixer for 10 sec.
9) Add **500 µl** of **absolute ethanol** and mix immediately by using pipette. **Do not centrifuge**.
10) Transfer the sample to the *AccuPrep*® Binding Column-III in a 2 ml collection tube, close the lid and centrifuge at **≥14,000 rpm** for **20 sec**.
11) Discard the flow-through from the collection tube and reuse the collection tube.
12) Add **700 µl** of **RWA1 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
13) Discard the solution from the collection tube and reuse the collection tube.
14) Add **500 µl** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
15) Discard the solution from the collection tube and reuse the collection tube.
16) Add **500 µl** of **RWA2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **2 min**.
17) Discard the solution from the collection tube and reuse the collection tube.
18) Centrifuge once more at **14,000 rpm** for **1 min** to completely remove ethanol, and check that there is no droplet clinging to the bottom of

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the *AccuPrep*® Binding Column-III tube.

19) Transfer the *AccuPrep*® Binding Column-III to a new 1.5 ml tube for elution, add **50~200 µl** of **ER Buffer** onto *AccuPrep*® Binding Column-III, and wait for at least 1 min at RT (15~25˚C).
20) Centrifuge at **10,000 rpm** for **1 min** to elute.

## (III) RNA Extraction from gram-positive bacteria

1) **Weigh 50 mg acid-washed glass beads** (150~600 µm) in a 2 ml tube (not supplied), for use in step 10.
2) Calculate the volume of bacteria culture (1 volume).
3) Add **0.5 volume** of **RS Buffer** into a tube (not supplied).
4) Add **1 volume** of **bacteria culture** to the tube and mix by vortex mixer for **5 sec. Incubate** for **5 min** at room temperature (15~20˚C).
5) Centrifuge at **7,500 rpm** for **10 min**.
6) Discard the supernatant from the tube.
7) Add **20 µl** of **Proteinase K** to the 100 µl of TE buffer containing lysozyme, and add the mixture to the tube.
8) Resuspend the pellet by pipetting and mix by vortex mixer for 10 sec. **Incubate** for **10 min** at room temperature (15~20˚C).
9) Add **700 µl** of **RB Buffer** to the tube and mix by vortex mixer for 10 sec.
10) Transfer the suspension into a 2 ml tube containing the acid-washed glass beads prepared in step 2. **Homogenize** the cells using bead beater (in case of using TissueLyser, homogenize the cells for **5 min** at **50 Hz**).
11) Centrifuge for **10 sec** at maximum speed. Transfer the supernatant into a new tube (not supplied).
12) Go to **step 9** of "RNA Extraction from gram-negative bacteria" in page 1 and follow the instructions accordingly.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.

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