This method allows bacterial RNA isolation from up to 3 ml LB culture.

1. **Grow Bacteria in LB media to log phase**. **(Do not use overnight culture.)**

2. Harvest no more than 3 ml culture (< 5 x 10^8 bacteria) by centrifugation at 4,000-5000 x g for 5 min at 4°C.

3. Discard medium and resuspend cells in 100µl Lysozyme/TE Buffer**. Add 10µl of (20 mg/mL) Proteinase K(not Supplied) Mix by vortexing at maxi speed for 30 seconds.**

   Note: The amount of enzyme required and/or the incubation time may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis**.**

4. **Incubate at 30°C for 10 minutes. Incubate on a shaker-incubator or vortex 20 seconds for every 2 minutes during incubation.**

5. Add 350 µl BRK lysis buffer and 25-40 mg glass beads to the sample and vortex vigorously for 5 minutes. Centrifuge for 5 minutes at maximum speed in a micro-centrifuge.

   Note: Ensure β-mercaptoethanol (β-ME) is added to BRK Lysis Buffer (20 µl/ml) before use.

6. **Transfer 400µl of the supernatant into a new 1.5 ml centrifuge tube.**

7. Add 280 µl absolute ethanol (96-100%) to the lysate and mix well by vortexing at maxi speed for 15 seconds.

8. Apply sample, including any precipitate that may have formed, to a HiBind® RNA mini column inserted in a 2 ml collection tube. Centrifuge for 30 seconds at 8,000-10,000 x g. Reuse the collection tube for next step.
9. Add 400µl RNA Wash Buffer I to the column. Centrifuge at 10,000 x g for 2 minutes. Discard the flow-through and collection tube. If on-membrane DNase I digestion is desired, proceed to Step 10, otherwise go to Step 12.

10. **DNase I Digestion (Optional)**
    Since HiBind® RNA resin and spin-column technology actually removes most of DNA without the DNase I treatment, it is not necessary to do DNase digestion for most downstream applications. However, certain sensitive RNA applications might require further DNA removal. Follow the steps below for on-membrane DNase I digestion. (See DNase I manual, Product No. E1091 for detailed information.)

    a. For each HiBind® RNA column, prepare the DNase I digestion reaction mix as follows:

OBI DNase I Digestion Buffer	73.5 µl
RNase-free DNase I (20 Kunitz unites/µl)	1.5 µl
Total volume	75 µl

Note:

- **DNase I is very sensitive and is subject to physical denaturation; so do not vortex the DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation.**
- **OBI DNase I digestion buffer is supplied with OBI RNase-free DNase I set**.
- **Standard DNase buffers are not compatible with on-membrane DNase I digestion.**

b. Dry column by spinning an additional 30 seconds, then pipette 75 µl of the DNase I digestion reaction mix directly onto the surface of the HiBind® RNA membrane in each column. Make sure to pipette the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mix sticks to the wall or the O-ring of the HiBind® RNA column.

c. Incubate at room temperature(25-30°C) for 15 minutes.

11. Place HiBind® RNA Mini column in a clean 2 ml collection tube, and add 500 µl RNA Wash Buffer I. **(If on-membrane DNase I digestion was performed in the previous step, allow wash buffer to soak column at least 5 minutes before proceeding).** Centrifuge as above and discard flow-through.

12. Place HiBind® RNA Mini-column in a new 2 ml collection tube (provided). **Add 500 µl RNA Wash Buffer II and spin for 30 seconds at 10,000 x g.** Discard flow-through and reuse the collection tube.

13. **Add 500 µl RNA Wash Buffer II to column and centrifuge for 30 seconds at 8,000-10,000 x g to wash again**. Discard the flow-through and reuse the collection tube.

14. Using the same collection tube, dry the column by spinning for 3 minutes at 8000-10,000 x g to dry the column. **Note: Drying the HiBind® RNA Mini column is very important for removal of residual ethanol that will otherwise interfere with downstream applications.**

15. Transfer HiBind® RNA Mini column to a new RNase free 1. 5 ml collection tube (not supplied) and add 50-100 µl DEPC water directly onto the HiBind® membrane. Centrifuge for 1 minute at 8,000-10,000 x g to elute. Repeat if the expected RNA yield is more than 60 µg.