# Purification of miRNA from Whole Blood using Hybrid-R™ miRNA Kit

This protocol is especially designed for isolation of miRNA from various liquid samples.

**Preparation**
- Buffer RiboEx™ LS 50 ml (Cat. No. 302-932)
- 1.5 ml microcentrifuge tube

1. Prepare 750 µl RiboEx™ LS (not provided) in a 1.5 ml microcentrifuge tube (not provided).

2. Add 250 µl blood sample to the 1.5 ml microcentrifuge tube and vortex vigorously.

3. Incubate for 5 min at room temperature.

4. Add 0.2 ml chloroform. Shake vigorously for 15 sec and let it stand for 2 min at room temperature.
   Alternatively, 0.1 ml of BCP (1-bromo-3-chloropropane) can be used in place of chloroform.

5. Centrifuge at 12,000 x g for 15 min at 4°C.
   The mixture will be separated into three phases; a lower layer, an interphase, and a colorless upper aqueous layer.
   The upper aqueous volume is about 450 µl.
   Centrifugation at temperatures >8°C may cause some DNA to partition in the aqueous phase.

6. Transfer the aqueous phase (approximately 350 µl) to a 1.5 ml microcentrifuge tube (not provided).
   To obtain higher yield, transfer all aqueous phase.

7. Transfer up to 700 µl of the mixture to a Column Type B mini (red ring).
   Repeat step 6~7 when the mixture volume is larger than 700 µl.

8. Centrifuge at ≥10,000 x g for 30 sec at room temperature. Transfer the mini column to a new 2 ml collection tube (provided), and store at room temperature. Use the pass-through for small (micro) RNA purification.
   Make sure that no mixture remains in the mini column after centrifugation. If the residual mixture has remained, centrifuge again at higher speed until all of the solution has pass-through.
   After this step, large RNA bind to mini column and small (micro) RNA exist in the pass-through.

9. Go on to step 9 of Hybrid-R™ miRNA protocol (page 14) for small RNA purification.
   9-1. Go on to step 21 of Hybrid-R™ miRNA protocol (page 16) for large RNA purification.





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