

SanPrep Column MicroRNA Mini-Prep Kit

Description

SanPrep Column microRNA Mini-Preps Kit is used for the isolation of miRNA, siRNA, snRNA (<200 nt) and total RNA. This miRNAExtractor performs well with both small and large quantities of tissue and cells from human, animal, plant or bacterial origin. The kit is a new addition to our catalogue that provides a rapid and efficient method for purifying and enriching miRNA along with other small RNAs, allowing researchers to obtain high-quality miRNA directly from cells or tissues as rapidly and simply as a total RNA prep.

Features

- Optimized for purification of microRNA and other small RNA directly from diverse biological sources.
- Fast and efficient. The entire procedure can be completed in 30 minutes.
- High-purity and high-yield microRNA. The purified microRNA can be used subsequently in many applications.

Contents

Components	SK8811 (50 Preps)
miRNAExtractor	60 ml
RPE Solu?on	12 ml*
RNase-free water	5 ml
Spin Column TR with Collec?on Tube	50
Spin Column MR with Collec?on Tube	50
Protocol	1

*RPE Solution is supplied in a concentrated form, before use, add 4 volumes of 100% ethanol into concentrated RPE solution and mix well.

SanPrep Column MicroRNA Mini-Prep Kit



Storage

Shipped at room temperature, store at 2-8°C and protect from light. miRNAExtractor is harmful on contact with skin or if swallowed—please avoid contact with eyes, skin, and clothes. Wash thoroughly after handling and seek professional help if necessary.

Before Starting

1. Materials Required by User: RNase-Free Ethanol (96-100%), Chloroform.
2. RPE Solution is supplied in a concentrated form, before use, add 4 volumes of 100% ethanol into concentrated RPE solution and mix well.
3. Care must be taken when working with RNA. It is important to maintain an RNase-Free environment starting with RNA sample preparation and continuing through purification and analysis. Use RNase free tubes, tips, and gels. Wear gloves at all times.
4. Homogenized cell lysates (before adding chloroform) can be stored at -70°C for several months.

Sample Preparation

1. Plant tissue: Grind ~50-100 mg plant tissue to a fine powder in liquid nitrogen. Transfer the powder to a 1.5 ml RNase-free centrifuge tube. Add 1 ml miRNAExtractor, mix immediately by inverting the tube.
2. Animal tissue: Cut the tissue into small pieces. Add 1 ml miRNAExtractor per every ~25-50 mg tissue. Homogenize for 30 seconds using a homogenizer.
3. Adherent cells: Discard the culture medium carefully. Add 1 ml miRNAExtractor per ~10 cm² cultured cells (approximately 10⁷ cells). Mix gently by pipetting up and down.
4. Suspension cells: Collect cells by centrifuge, discard the supernatant. Add 1 ml miRNAExtractor for 10⁷ cells, mix gently by pipetting. The amount of cells should not be exceed 1 × 10⁷ for fibroblasts or carcinoma cell.

SanPrep Column MicroRNA Mini-Prep Kit



5. **Blood:** Add 0.5 ml fresh anticoagulated whole blood to a 1.5 ml RNase-free centrifuge tube. Centrifuge at 10,000 x g for 2 minutes at room temperature, discard the supernatant (plasma). Add 0.5 ml RNase-free Water and mix by inverting. Centrifuge at 10,000 x g for 2 minutes at room temperature, discard the supernatant. Add 1 ml miRNAExtractor, mix by inverting the tube immediately.

Procedure 1 (Isolation of Total RNA):

1. Incubate samples (with miRNAExtractor) at room temperature for 5-10 minutes to ensure samples are completely lysed.
2. Add 0.2 ml chloroform, vortexing for 30 seconds. Centrifuge at 12,000 × g for 10 minutes at 4°C.

NOTE: After centrifugation, the sample separates into 3 phases. The upper, colorless, aqueous phase contains the RNA.

NOTE: After centrifugation, a white inter-phase containing DNA and protein may appear. Avoid transferring this layer to reduce DNA contamination.

3. Transfer the supernatant (approximately 540 µl) into a clean 1.5 ml RNase-free centrifuge tube, add 1.5 volumes (usually 810 µl) of 100% ethanol and mix thoroughly by pipetting up and down several times.

NOTE: Do not centrifuge. A precipitate may form after addition of ethanol, but this will not affect the yield.

4. Transfer the solution to the Spin Column TR, centrifuge at 12,000 × g for 2 minutes, discard the flow-through.

NOTE: If the volume of lysate exceeds 750 µl, add 750 µl to the column, centrifuge for 30 seconds and discard liquid. Add the remaining lysate to the same column and centrifuge again as above.

SanPrep Column MicroRNA Mini-Prep Kit



5. Add 0.5 ml of RPE Solution to the Spin Column TR, centrifuge at $12,000 \times g$ for 30 seconds, discard the flow-through.

NOTE: Check the label to ensure the RPE Solution has been diluted with ethanol.

6. Repeat step 5 once.
7. Centrifuge the column at $12,000 \times g$ for 30 seconds.

NOTE: This step is very important to remove the residual ethanol thoroughly.

8. Place the column to a new 1.5 ml centrifuge tube, add 30-50 μ l RNase-free Water, and let it stand for 2 minutes. Centrifuge at $12,000 \times g$ for 30 seconds, save the eluted RNA solution at -80°C .

Procedure 2 (Isolation of microRNA)

1. Incubate samples (with miRNAExtractor) at room temperature for 5-10 minutes to ensure samples are completely lysed.
2. Add 0.2 ml chloroform, vortexing for 30 seconds. Centrifuge at $12,000 \times g$ for 10 minutes at 4°C .

NOTE: After centrifugation, the sample separates into 3 phases. The upper, colorless, aqueous phase contains the RNA.

NOTE: After centrifugation, a white inter-phase containing DNA and protein may appear. Avoid transferring this layer to reduce DNA contamination.

3. Transfer the supernatant (approximately 540 μ l) into a clean 1.5 ml RNase-free centrifuge tube, add 1/3 volumes (usually 180 μ l) of 100% ethanol and mix thoroughly by pipetting up and down several times.

NOTE: Do not centrifuge. A precipitate may form after addition of ethanol, but this will not affect the yield.

SanPrep Column MicroRNA Mini-Prep Kit



4. Transfer the solution to the Spin Column TR centrifuge at $12,000 \times g$ for 2 minutes, transfer the flow-through (usually 690 μ l) to a new 1.5 ml RNase-free centrifuge tube.
5. Add 2/3 volumes of 100% ethanol (e.g. if 690 μ l flow-through, add 460 μ l ethanol) and mix thoroughly by pipetting up and down several times. Transfer the solution to the Spin Column MR and centrifuge at $12,000 \times g$ for 2 minutes, discard the flow-through.

NOTE: If the volume of lysate exceeds 750 μ l, add 750 μ l to the column, centrifuge for 30 seconds and discard liquid. Add the remaining lysate to the same column and centrifuge as above.

6. Add 0.5 ml of RPE Solution to the Spin Column, centrifuge at $12,000 \times g$ for 30 seconds, discard the flow-through.

NOTE: Check the label to ensure RPE Solution was diluted with ethanol.

7. Repeat step 6 once.
8. Centrifuge the column at $12,000 \times g$ for 30 seconds.

NOTE: This step is very important to remove the residual ethanol thoroughly.

9. Put the column to a new 1.5 ml centrifuge tube, add 30-50 μ l RNase-free Water. Let it stand for 2 minutes. Centrifuge at $12,000 \times g$ for 30 seconds, save the eluted RNA solution at -80°C .



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NOT INTENDED FOR HUMAN OR ANIMAL USE