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TaqMan® miRNA ABC Purification Kit

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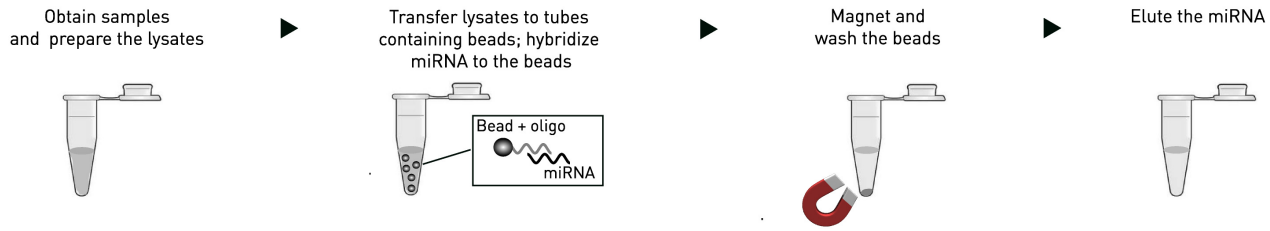
This quick reference is intended for advanced users who are familiar with the procedures in the *TaqMan® miRNA ABC Purification Kit User Guide* (Part no. 4473439).

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® miRNA ABC Purification Kit User Guide.* For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow summary



Prepare the buffers

Add 6 mL of Lysis Buffer to the ABC Buffer, and 7 mL of 100% ethanol to Wash Buffer 2. Store buffers at room temperature (15°C to 30°C).

Prepare the samples

- For blood type samples, follow standard collection procedures for whole blood, plasma, or serum. Use samples that are fresh or stored at –86°C to –68°C in citrate, EDTA, or heparin. Blood can be collected directly into Tempus™, PAXgene, or other blood lysis tubes (blood lysate).
- For cultured cells, harvest according to standard tissue culture practices. Wash cells twice with 1X PBS to remove media, and collect as a cell pellet.
- For FFPE tissue:
  - If tissue is on a slide, wash once for 3 minutes with xylene, then air dry completely prior to lysis.
  - If tissue is in a 1.5-mL LoBind tube, wash once with 1 mL xylene and vortex for 10 seconds. Centrifuge tissue at full speed (15,000–20,000 × g) for 2 minutes, then remove xylene. Repeat wash and centrifuge steps with 1 mL 100% ethanol, then air dry completely prior to lysis.

Prepare the lysates

The following table lists the reagents and volumes to use for respective sample types.

Sample type	Sample amount	Reagent	Reagent volume
Whole blood	10 µL	Lysis Buffer	20 µL
		ABC Buffer	120 µL
Blood lysate	30 µL	ABC Buffer	120 µL
Plasma	50 µL	ABC Buffer	100 µL
Serum	50 µL	ABC Buffer	100 µL
Cultured cells	10–1 × 10^6 cells in 50 µL of 1X PBS	Lysis Buffer	150 µL
Solid tissue	1–10 mg, homogenized if necessary	Lysis Buffer	100 µL
		ABC Buffer	50 µL
FFPE tissue	5–10 µ thick × 3–5 mm in diameter	Lysis Buffer	100 µL
		ABC Buffer	50 µL

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Sample type	Sample amount	Reagent	Reagent volume
Saliva†	50 µL	Lysis Buffer	100 µL
Urine†	50 µL	ABC Buffer	100 µL

† Centrifuge for 15 minutes at 500–2000 × g to remove debris.

1. In a 1.5-mL microcentrifuge tube (not provided), combine sample with Lysis Buffer if needed, and vortex for 30 seconds to lyse.
2. Centrifuge briefly to collect the liquid at the bottom of the tube(s).
3. Add ABC Buffer to lysate if needed, vortex for 30 seconds to mix, then centrifuge briefly.
4. (*Optional*) Add 2 µL of 1 nM external control miRNA into the prepared sample(s),vortex to mix, then centrifuge briefly.

Prepare the Human Panel Beads

1. Vortex the beads thoroughly to suspend in solution.
2. Sonicate (*recommended*), or vortex the beads at maximum speed, for 1 minute using an ultrasonic water bath or vortex mixer. If beads are sonicated, mix well by vortexing.
3. Centrifuge briefly, then aliquot 80 µL of beads into a LoBind 1.5-mL microcentrifuge tube for each sample.
4. Place the beads on a magnetic rack for 1 minute and remove the supernatant. Proceed to "Hybridize the samples" for all sample types, except for FFPE and solid tissues.
5. For FFPE and solid tissues *only*, resuspend the beads in 50 µL ABC Buffer.

Hybridize the samples

1. Transfer sample lysates, except FFPE and solid tissues, into prepared Human Panel Bead tube(s). For FFPE and solid tissue lysates, transfer beads in ABC Buffer to the tube(s) containing the lysates.
2. Vortex the beads until suspended in solution, then centrifuge briefly.
3. Hybridize for 40 minutes by shaking the tube(s) in a 30°C Thermomixer at 1200 rpm (*recommended*), or by using a vertical tube rotator at 20°C to 35°C.

Wash the samples

1. Place the beads on a magnetic rack for 1 minute and carefully discard the supernatant without disturbing the beads.
2. Add 100 µL Wash Buffer 1. Vortex briefly, then centrifuge briefly and incubate for 1 minute at room temperature. Place the beads on a magnetic rack for 1 minute and remove the supernatant.
3. Add 100 µL Wash Buffer 2. Vortex briefly, then centrifuge briefly and incubate for 1 minute at room temperature. Place the beads on a magnetic rack for 1 minute and remove the supernatant.
4. Repeat step 3 for a third wash.
5. Centrifuge briefly, then place the tube(s) on a magnetic rack for 1 minute. Remove any residual liquid using a fine pipette tip.

Elute the samples

1. Add 100 µL Elution Buffer and vortex. Centrifuge briefly.
2. Elute for 3 minutes by using a 70°C Thermomixer at 1200 rpm (*recommended*), or by using a standard 70°C incubator.
3. Immediately place the tube(s) on a magnetic rack for 1 minute.
4. Carefully transfer the supernatant containing the miRNA sample(s) into clean 1.5-mL LoBind tube(s).
5. (*Optional*) Add 500 µL of Nuclease-free Water to dilute the sample(s) for a 96-well reverse transcriptase reaction.
6. Place the sample tube(s) on ice. Store the miRNA sample(s) at –86°C to –68°C, if not used immediately.

Analyze isolated miRNA

To analyze miRNA isolated by the TaqMan® miRNA ABC Purification Kit, perform reverse transcription using specific reverse transcriptase primers followed by qPCR with the appropriate primer set. Refer to the *TaqMan® miRNA ABC Purification Kit User Guide* for more details.

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