

# TaqMan® miRNA ABC Purification Kit

## For sensitive and specific miRNA expression analysis

In about 75 minutes or less, you can purify specific miRNAs from blood, serum, or plasma without hazardous chemicals. The TaqMan® miRNA ABC (Anti-miRNA Bead Capture) Purification Kit lets you obtain high-quality miRNA for sensitive and specific miRNA expression analysis. It uses Dynal® magnetic beads conjugated to anti-miRNA oligonucleotides to purify miRNAs from difficult sample types. Choose Human Panel A or Human Panel B options to purify miRNAs corresponding to a total of 754 specific miRNA assays contained in TaqMan® Array Human MicroRNA A or B Cards.

### Benefits

- No need for hazardous chemicals such as phenol, chloroform, or guanidine isothiocyanate
- Purify miRNA from a broad range of sample types: blood, serum, plasma, FFPE, solid tissues, cultured cells, saliva, or urine
- Volumes typically range from 10  $\mu$ L for blood samples to 50  $\mu$ L for serum and plasma samples
- RT-qPCR inhibitors commonly found in blood-related samples are easily washed away
- Simple and fast workflow using oligonucleotide-conjugated Dynal® magnetic beads typically purifies miRNA in about 75 minutes or less



### Simple and fast miRNA purification without hazardous chemicals

The TaqMan® miRNA ABC Purification Kit contains buffers and magnetic beads for isolation of specific microRNAs (miRNA) from small inputs of many human sample types, including blood, serum, plasma, FFPE samples, solid tissues, cultured cells, saliva, and urine. After sample lysis, the entire purification process occurs within a single tube and involves only 5 simple steps: hybridization of miRNA to the beads, three wash steps, and

miRNA elution (Figure 1). Typical input volumes range from 10  $\mu$ L for whole blood to 50  $\mu$ L for serum and plasma samples. Because miRNAs are captured by the magnetic beads, RT-PCR inhibitors commonly found in blood-related samples are easily washed away. The process uses no hazardous chemicals such as phenol, chloroform, or guanidine isothiocyanate, and the entire procedure is completed in about 75 minutes.

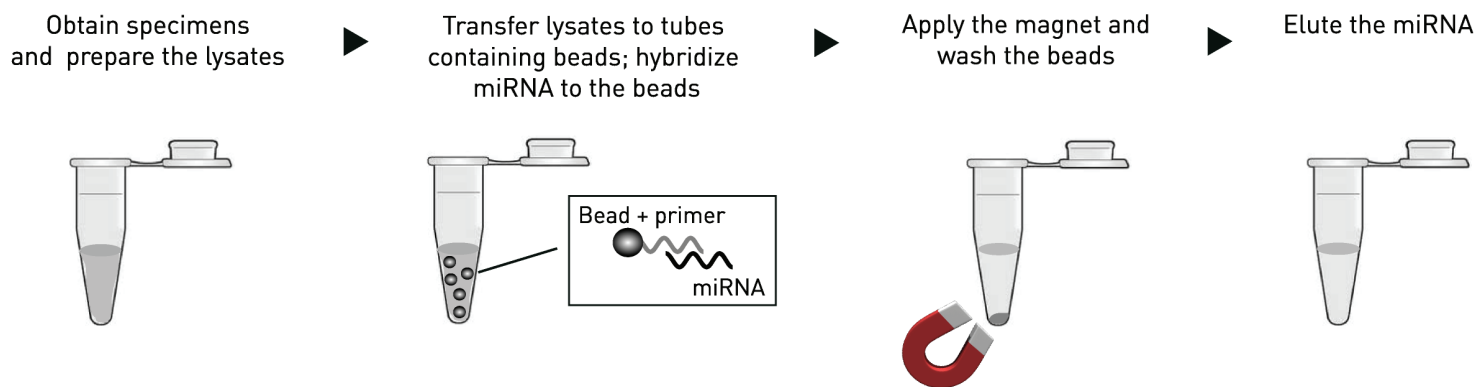


Figure 1. Workflow using the TaqMan® miRNA ABC Purification Kit.

### Human Panel A and Human Panel B

The TaqMan® miRNA ABC Purification Kit is offered in two configurations: Human Panel A and Human Panel B. The beads in each kit are superparamagnetic Dynabeads® covalently bound to a unique set of 377 anti-miRNA oligonucleotides for each panel that correspond to the assays contained in the TaqMan® Array Human MicroRNA A and B Cards. The miRNA isolation process relies on hybridization of miRNAs to the corresponding anti-miRNA oligonucleotides attached to the beads. In addition to the human miRNAs (total of 754), both Human Panel A and Human Panel B beads contain oligonucleotides that can isolate a set of six exogenous and three endogenous control miRNAs. Complete lists of miRNAs isolated by the panels can be found in the *TaqMan® miRNA ABC Purification Kit User Guide* (Cat. No. 4473439).

### Sensitive and reproducible miRNA analysis

The miRNAs isolated using the TaqMan® miRNA ABC Purification Kit are ready for conversion to cDNA and downstream analysis by quantitative PCR using either single-tube (individual) TaqMan® miRNA assays, the TaqMan® Array Human miRNA A and B Cards, or the TaqMan® OpenArray® Human miRNA Panel. The TaqMan® miRNA ABC Purification Kit is able to purify miRNA with greater reproducibility and often with greater yield than the leading competitor's miRNA purification kit. As shown for a panel of TaqMan® miRNA assays with miRNA isolated from plasma (Figure 2), lower  $C_t$  values (hence higher miRNA yields) were obtained for several of the assays using the TaqMan® miRNA ABC Purification Kit, and all assays exhibited greater reproducibility.

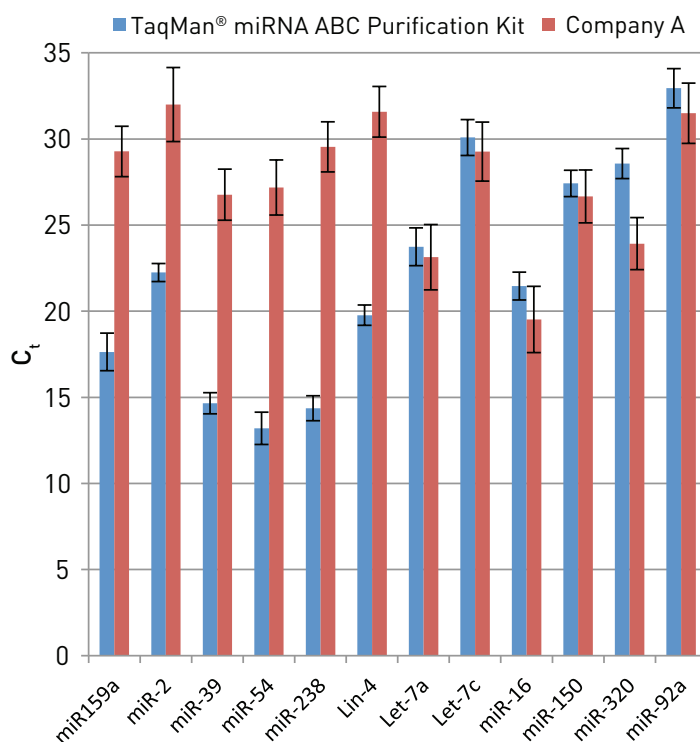
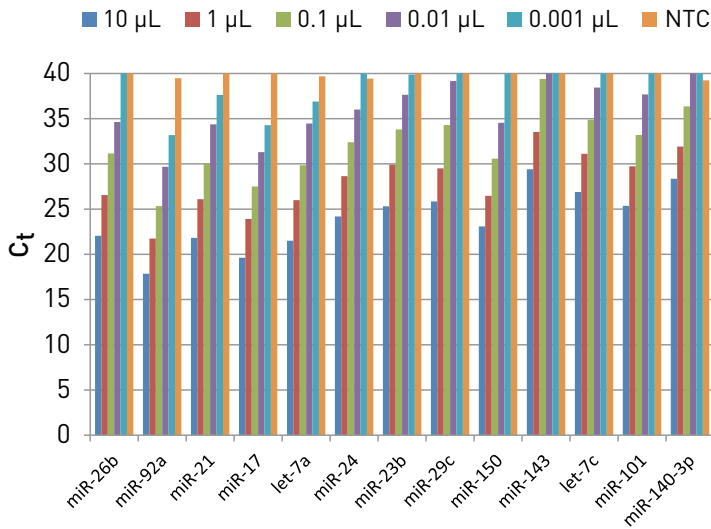


Figure 2. Robust and reliable miRNA quantification from plasma using the TaqMan® miRNA ABC Purification Kit. MicroRNA was purified from seven identical plasma samples (50  $\mu$ L each) using the TaqMan® miRNA ABC Purification Kit and the miRNA purification kit from Company A. Single-replicate RT and qPCR assays were performed for each sample, and each data point represents the average of the seven sample prep-to- $C_t$  replicates. In addition to lower  $C_t$  values for many of the assays, all assays exhibited greater reproducibility with the TaqMan® miRNA ABC Purification Kit. The average standard deviation using the TaqMan® miRNA ABC Purification Kit (0.85) is 2-fold lower than when using the competitor's kit (1.66).

### Reproducible purification from small sample sizes

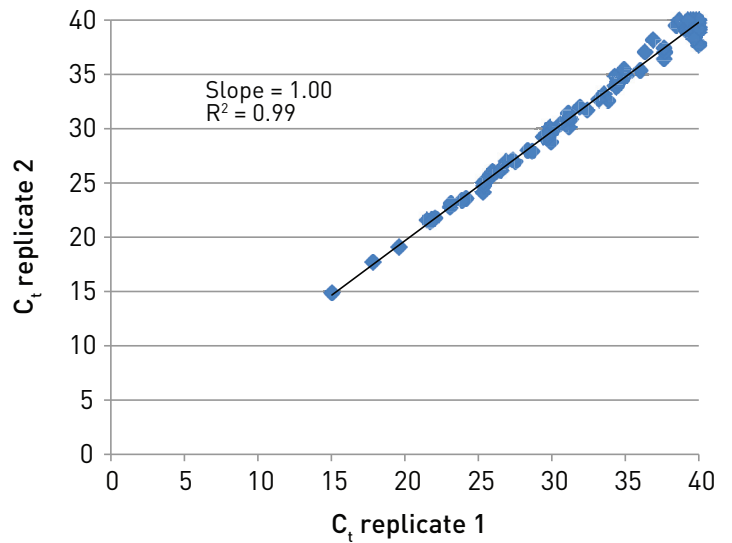
One of the major benefits of the TaqMan® miRNA ABC Purification Kit is the ability to purify miRNA from small amounts of sample. For most fluid samples (serum, plasma, saliva, and urine) only 50 µL of input is required, and only 10 µL is required for whole blood. To demonstrate this capability, microRNA was purified from 10-fold serial dilutions of whole blood, starting with 10 µL, and tested with a panel of 13 miRNA assays (Figure 3). With the majority of assays, miRNA can be detected with the equivalent of 0.1 µL of whole blood. Duplicate samples were processed in parallel, and a pairwise comparison of the data shows reproducible miRNA recovery from both low and abundant miRNA levels (Figure 4).



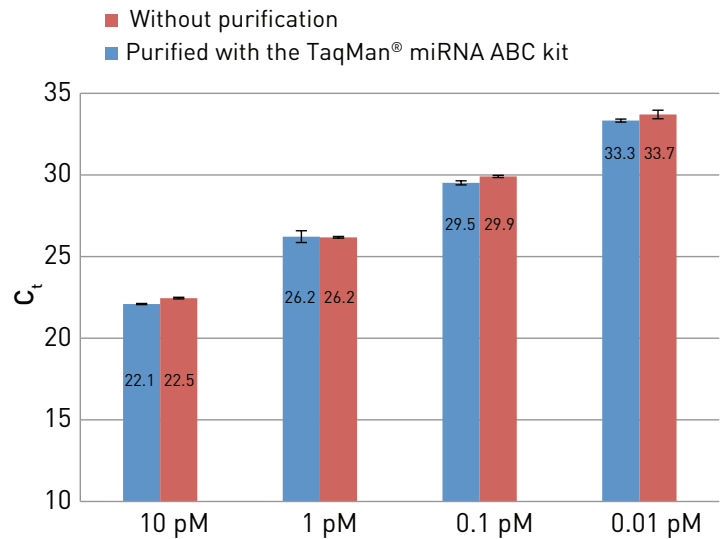
**Figure 3. MicroRNA from small quantities of whole blood.** MicroRNA was purified from 10-fold serial dilutions of whole blood, starting with 10 µL, and tested with a panel of 13 miRNA assays. MicroRNA was detected at  $C_t < 35$  in the equivalent of as little as 0.1 µL of whole blood, for 10 out of 13 assays.

### Excellent recovery

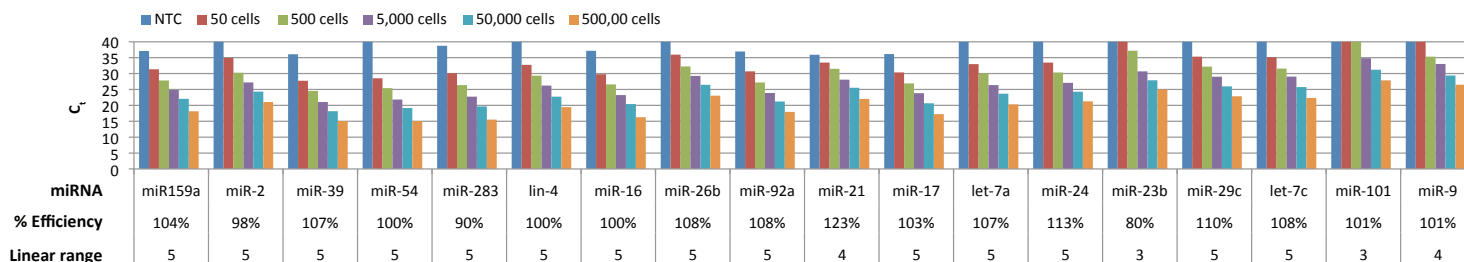
The magnetic beads have a theoretical capacity to capture 128–380 fmol of each miRNA. A spike-in experiment was performed with an artificial template to assess the miRNA recovery rate (Figure 5). Plasma samples were spiked with different concentrations of synthetic nonhuman miRNA cel-mir-238 either before or after purification using the TaqMan® miRNA ABC Purification Kit. The difference in the resulting  $C_t$  values between the purified and nonpurified cel-mir-238 miRNA is less than 0.4 at each concentration, signifying an estimated recovery rate between 75% and 100%.



**Figure 4. Reproducible results from small-volume samples.** Duplicate miRNA samples were prepared from the serially diluted blood described in Figure 3, and the pairwise comparison of the  $C_t$  values from the duplicate samples is shown. The results demonstrate the consistent and reproducible miRNA recovery from both low and abundant miRNAs.



**Figure 5. Spike-in test demonstrates excellent recovery rates.** Four different concentrations of synthetic nonhuman miRNA cel-mir-238 were spiked into 50 µL plasma samples either before or after purification using the TaqMan® miRNA ABC Purification Kit. Duplicate reverse transcription and qPCR reactions were performed for each sample. The difference in  $C_t$  is less than 0.4 at each concentration, resulting in an estimated recovery rate of 75–100%.



**Figure 6. Linear range and sensitivity with tissue culture cells.** A panel of 18 miRNA TaqMan<sup>®</sup> assays was performed with miRNA purified from samples containing 10-fold dilutions of tissue culture cells, ranging from 500,000 to 50 cells. For the majority of the assays (15/18), miRNA was detected from just 50 cells. In addition, the majority of the assays exhibited a linear dynamic range of 5 logs (14/18) and PCR efficiencies of 90–110% (15/18).

### Sensitive miRNA detection and broad dynamic range

The TaqMan<sup>®</sup> miRNA ABC Purification Kit is able to purify miRNA from a wide input range without compromising sensitivity or PCR efficiency (Figure 6).

### Automation-ready for high-throughput applications

The TaqMan<sup>®</sup> miRNA ABC Purification Kit is compatible with robotic systems and downstream applications that enable high-throughput miRNA expression profiling experiments. When coupled with the MagMAX<sup>™</sup> Express-96 Magnetic Particle Processor, miRNA can be extracted from 96 samples simultaneously in approximately 1 hour. Once purified, the miRNA can be analyzed using the QuantStudio<sup>™</sup> 12K Flex Real-Time PCR System with OpenArray<sup>®</sup> plates to generate over 43,000 data points from up to 48 samples, typically in just one day.

### A complete miRNA analysis workflow solution

The TaqMan<sup>®</sup> miRNA ABC Purification Kit enables a seamless workflow solution for miRNA analysis. Unlike other sample preparation methods that produce a mixed population of RNAs, the miRNA isolated with the TaqMan<sup>®</sup> miRNA ABC Purification Kit is a pure miRNA population that is matched and ideally suited for use with the Life Technologies family of TaqMan<sup>®</sup> miRNA assay products, to generate highly sensitive and specific miRNA expression and profiling results.

### Ordering information

Product	Quantity	Cat. No.
TaqMan <sup>®</sup> miRNA ABC Purification Kit-Human Panel A	40 purifications	4473087
TaqMan <sup>®</sup> miRNA ABC Purification Kit-Human Panel B	40 purifications	4473088
TaqMan <sup>®</sup> miRNA ABC Purification Kit-Human Panels A & B	40 purifications	4468071

Learn more at [lifetechnologies.com/abcmirna](http://lifetechnologies.com/abcmirna)

