# miND

miND® spike-in

Cat no: KT-041-MIND

Instruction Manual v2.0

October 2025

TAmiRNA

stability for life.

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Further information and technical notes can be found at **https://www.tamirna.com/mind-spike-in-controls-for-small-rna-sequencing-tamirna/**

## Revision history

| Date | Revision |
|---|---|
| 09/2022 | 1.0 |
| 02/2023 | 1.1 |
| 04/2023 | 1.2 |
| 05/2023 | 1.3 |
| 06/2023 | 1.4 |
| 05/2024 | 1.5 |
| 10/2025 | 2.0 |

# 1

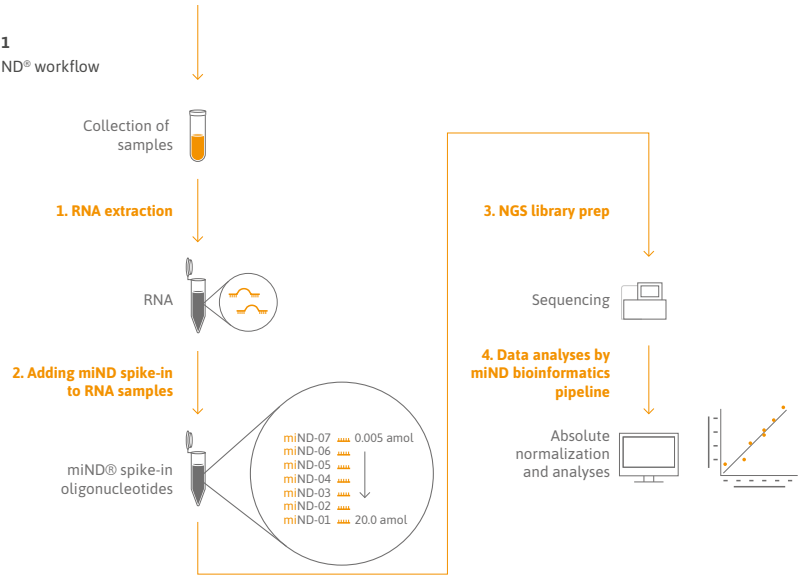
# Product Summary

# Intended Use

The miND® (**mi**croRNA **N**GS **D**ata Analysis) spike-in has been developed for **quality control** of small RNA-sequencing data and **absolute quantitation** of microRNAs in biofluids as well as cells and tissues across a broad range of species (Khamina et al. 2022). The miND® spike-in is added to an RNA sample during library preparation. The miND® spike-in consists of seven oligonucleotides that are provided in a specific ratio to cover the broad concentration range of endogenous small RNAs. A unique design of the miND® spike-in reduces sequencing bias and ensures precise quantitation of small RNA (Lutzmayer, Enugutti, and Nodine 2017).

The miND® spike-in sequences are detected in the NGS data along with the endogenous small RNAs. Read counts of the miND® spike-in and endogenous miRNAs are used to calculate absolute concentrations (amol/µL or molecules/µL). This conversion can either be achieved by using our miND® NGS data pipeline (Figure 1) (Diendorfer et al. 2022) or by the incorporation of the provided scripts in an already established NGS data analysis workflow.

**Figure 1**
The miND® workflow



# miND® spike-in Design Features

Each of the seven miND® spike-in consists of a unique 13-nucleotide core sequence that is flanked by four randomized nucleotides on the 5' and 3' ends (Table 1) resulting in 65,536 different RNA oligonucleotides per core sequence. The incorporation of randomized nucleotides is intended to reduce ligation bias, thereby improving the accuracy of small RNA quantification. In addition, all miND® spike-in oligonucleotides carry a 5' phosphate group to ensure compatibility with standard small RNA library preparation protocols.

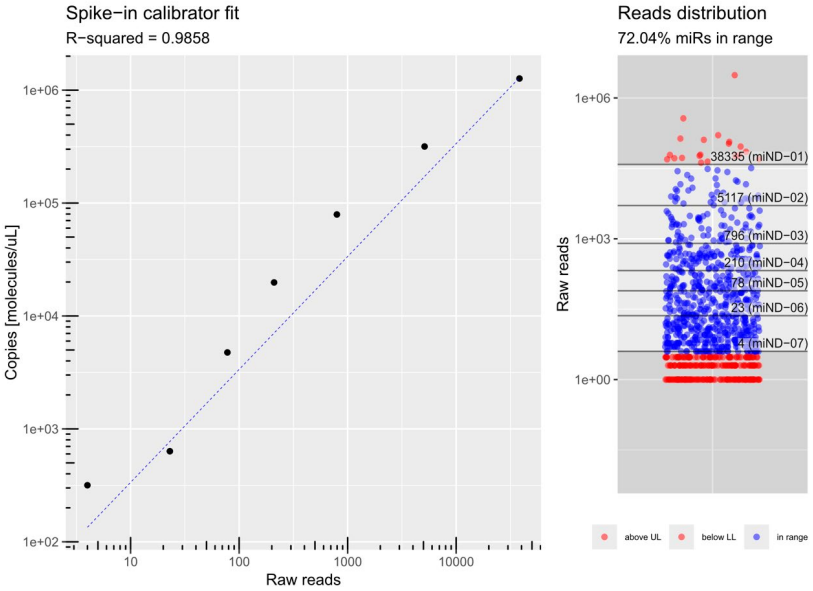
**Table 1**
The miND® spike-in core sequences

| Oligo | Sequence (5' - 3') | Molar amount (amol) in 1 µL of miND spike-in |
|---|---|---|
| **miND-01** | (N)(N)(N)(N)ACGAUCGGCUCUA(N)(N)(N)(N) | 20 |
| **miND-02** | (N)(N)(N)(N)UGAACGUCCGUAC(N)(N)(N)(N) | 5 |
| **miND-03** | (N)(N)(N)(N)UCUCGCGCGCGUU(N)(N)(N)(N) | 1.25 |
| **miND-04** | (N)(N)(N)(N)CGAGUAAUGAACG(N)(N)(N)(N) | 0.3125 |
| **miND-05** | (N)(N)(N)(N)GCUACACACGUCG(N)(N)(N)(N) | 0.075 |
| **miND-06** | (N)(N)(N)(N)UAUUCGCGGUGAC(N)(N)(N)(N) | 0.01 |
| **miND-07** | (N)(N)(N)(N)ACCUCCGUUUACG(N)(N)(N)(N) | 0.005 |

The miND® spike-in provides:

- **Quality control** for small RNA sequencing experiments by verifying the dynamic range and sensitivity of the assay.
- **Absolute quantitation** of endogenous miRNAs by generating a linear regression model for concentration calculation.

**Figure 2**
The miND® spike-in calibrator fit and miRNA spike-in reads distribution



# Product

The miND® spike-in is available in following formulations:

**Table 2**
Product overview

| Product Number | Formulation | Size | Shipment Condition | Storage |
|---|---|---|---|---|
| **KT-041-MIND-96** | lyophilized | 96 reactions | room temperature | lyophilized: store at -20°C or -80°C after reconstitution: store at -80°C |
| **KT-041-MIND-48** | resuspended, ready-to-use | 48 reactions | dry ice | store at -80°C |

# Stability and Handling Guidelines

**Table 3**
Stability and handling

| | |
|---|---|
| **KT-041-MIND-96** | This format is shipped at ambient temperature. Upon arrival, store at –20 °C for short-term use or at –80 °C for long-term storage. After reconstitution, prepare single-use aliquots and store at –80 °C. |
| **KT-041-MIND-48** | This format is shipped on dry ice and should be stored immediately at –80 °C upon arrival. |
| **Shelf life:** | Both formulations remain stable for at least 24 months when stored under the recommended conditions. |
| **Handling:** | Keep the resuspended spike-ins on ice during handling and return promptly to -80 °C after use. Avoid repeated freeze-thaw cycles. Use nuclease-free, low-binding tubes for aliqutos. |

# 2

# Important Pre-Analytical Considerations

# Choice of Sample Type and Protocols

The miND® spike-in has been extensively tested and optimised for plasma samples under the following conditions:

- RNA extraction from 200 µL of plasma with either miRNeasy Mini kit (Qiagen, Cat. 217004) or Maxwell RSC miRNA Tissue kit (Promega, Cat. AS1460), according to the manufacturer's protocols.
- Library Preparation with the RealSeq®-Biofluids Plasma/Serum miRNA Library Kit for Illumina® sequencing (Cat. 600-00012, 600-00024, 600-00048) or the RealSeq®-Dual Biofluids Plasma/ Serum miRNA Library Kit for Illumina® sequencing (Cat. 700-00024, 700-00048)
- Illumina sequencing with a minimum read lengthof 50 bp, and a required sequencing depth of at least 7.5 million reads per sample.

For other sample types, RNA isolation kits, or small RNA-sequencing workflows, the following considerations should be taken into account:

- The yield and efficiency of small RNA isolation vary between different kits, and the choice of isolation method may impact downstream results.
- The library preparation protocol can influence miND® spike-in performance. If alternative kits are used, a pilot experiment with a small number of samples is recommended to confirm that the spike-in adequately covers the concentration range of endogenous small RNAs.
- The miND® spike-in core sequences have been mapped against the genomes of *Homo sapiens, Mus musculus, Bos taurus, Rattus norvegicus, Sus scrofa, Caenorhabditis elegans, Drosophila melanogaster* and *Danio rerio* showing minimal overlap. For use with other species, it is recommended to contact the TAmiRNA team (support@tamirna.com).
- To minimize potential lot-to-lot variability, it is recommended to use a single batch of the miND® spike-in throughout a project. For projects exceeding 96 samples, reconstitute multiple tubes, pool and mix thoroughly, then aliquot to ensure consistency.

# Best Practices for Working with RNA and RNA spike-ins

- Maintain an RNase-free environment.
- Clean surfaces with RNase decontamination solutions.
- Use molecular-grade nuclease-free water.
- Use fresh consumables and low-binding tubes.
- Use filter barrier tips and dedicated equipment for RNA.
- Spin down all reaction and sample tubes before opening to avoid contamination.

# 3

# Lab Protocol

# Essential components

| |
|---|
| miND® spike-in kit |

## Consumables and Instruments (Not Supplied by TAmiRNA)

| |
|---|
| Molecular grade nuclease-free water |
| Nuclease-free, low nucleic acid binding tubes (1.5 mL) |
| Nuclease-free, filter pipette tips |
| Vortex mixer |
| Calibrated pipettes |
| Centrifuge for <2 mL tubes |
| small RNA NGS library preparation kit |

# Protocol

## 1. miND® spike-in Preparation

| | |
|---|---|
| **For KT-041-MIND-96:** | Reconstitution |
| **Step 1** | Centrifuge the lyophilized spike-in at 3,000 x g for 2 minutes to collect the oligo pellet at the bottom. |
| **Step 2** | Add **115 µL nuclease-free water**. |
| **Step 3** | Mix thoroughly by vortexing briefly or by pipetting up and down 10 times. Incubate on ice for 20 minutes, then vortex again and centrifuge briefly. |
| **For KT-041-MIND-48:** | The spike-in is provided ready-to-use. Proceed directly with Step 4. |

## 2. Aliquoting and Storage

| | |
|---|---|
| **Step 4** | Prepare single-use aliquots. |
| **Step 5** | Store aliquots at -80°C until use. |

## 3. Addition to RNA Samples

| | |
|---|---|
| **Step 6** | Thaw a spike-in aliquot on ice. |
| **Step 7** | Mix gently and centrifuge briefly to collect the liquid. |
| **Step 8** | Add **1 µL spike-in** to each RNA sample. |
| **Step 9** | Immediately proceed with library preparation according to the manufacturer's protocol. |

## 4. Sequencing Recommendations

| | |
|---|---|
| **Step 10** | Sequence the final libraries with a read length ≥ 50 bp and a minimum depth of 7.5 million reads per sample to ensure optimal recovery. |

Notes

# Data Analysis

The absolute quantification of miRNAs using miND® spike-in requires the following steps:

- mapping and quantification of microRNAs and other small RNAs
- mapping and quantification of miND® spike-in
- calculation of absolute concentrations using a regression model

For easy analysis, a docker based toolkit is available on our public GitHub space: https://github.com/tamirna

Documentation, examples, and updated tools will be published there.

In addition, we have published our in-house miRNA analysis pipeline miND® (Diendorfer et al. 2022) that can be used for the whole process of miRNA mapping from raw NGS data.

# Troubleshooting

## Calibration QC

A sample passes calibration QC if all of the following apply:

- At least five miND® spike-in core sequences are detected.
- Linear model parameters can be calculated.
- R-squared is ≥ 0.95.

**Table 4**
Troubleshooting guide

| Problem | Possible Cause | Recommended Action |
|---|---|---|
| **No spike-in reads detected** | Oligo pellet was lost during reconstitution | Check that the tube was centrifuged according to protocol before adding nuclease-free water. |
| | Incorrect reconstitution volume used | Verify that the recommended volume of nuclease-free water was added. |
| | Degradation due to RNase contamination | Ensure handling is performed under RNase-free conditions and with nuclease-free consumables. |
| | Data pipeline not applied correctly | Confirm that the miND® data analysis pipeline was used for processing. |
| **Spike-in QC failed** | Fewer than five spike-in core sequences were detected. | Verify that spike-ins were added correctly and handled according to protocol; repeat the preparation if necessary. |
| | Insufficient sequencing depth | Ensure a minimum depth of 7.5 million reads per sample. |
| | Spike-in degradation from improper storage | Confirm storage at –80 °C and avoid repeated freeze–thaw cycles. |
| | Low ligation efficiency of library kit | Evaluate compatibility of the library preparation kit and consider testing with the recommended kits. |

If issues persist, please contact support@tamirna.com for technical assistance.

# 4

# Additional Information

# Related Services

TAmiRNA offers a broad range of high-quality RNA analysis services:

- **small RNA-sequencing:** TAmiRNA is highly experienced with microRNA and small RNA NGS analysis. As we mainly work with extracellular RNA in biomarker research, we have long-lasting experience with very low-input RNA samples.
- **RNA-sequencing:** TAmiRNA offers mRNA and whole transcriptome sequencing services to explore the messenger RNA (mRNA), long non-coding RNA (lncRNA) and circular RNA transcriptome in any biological sample type including biofluids.
- **RT-qPCR services:** TAmiRNA specializes in developing and applying RT-qPCR assays for any small or large RNA. Our microRNA platform leverages target-specific LNA-enhanced (locked nucleic acid) primers with unmatched specificity and sensitivity.
- **Extracellular vesicle characterization:** TAmiRNA offers end-to-end services for extracellular vesicle purification and characterization, including surface markers and cargo analysis. We support customers in measuring EV concentration, surface markers, and RNA, DNA, protein, and lipid content.

Read more about these services at https://www.tamirna.com/comprehensive-rna-analysis-cro-services-tamirna/

# Related Products

TAmiRNA offers diagnostic-use and research-use kits for analysis of microRNA biomarkers.
Read more about the products at www.tamirna.com

# Further Reading

Below you find a list of publications describing the development and application of the miND® spike-in:


1. Khamina, K. et al. A MicroRNA Next-Generation-Sequencing Discovery Assay (miND) for Genome-Scale Analysis and Absolute Quantitation of Circulating MicroRNA Biomarkers. Int. J. Mol. Sci. 2022,23,1226. https://doi.org/ 10.3390/ijms23031226
2. Diendorfer, A. et al. miND (miRNA NGS Discovery pipeline): a small RNA-seq analysis pipeline and report generator for microRNA biomarker discovery studies. F1000Research 2022,11:233,1226. https://doi.org/10.12688/f1000research.94159.1
3. Gutmann, C. et al. Association of cardiometabolic microRNAs with COVID-19 severity and mortality. Cardiovasc Res. 2022, 118(2):461-474. https://doi.org/10.1093/cvr/cvab338
4. Lutzmayer, Stefan, Balaji Enugutti, and Michael D. Nodine. 2017. 'Novel Small RNA Spike-in Oligonucleotides Enable Absolute Normalization of Small RNA-Seq Data'. Scientific Reports 7 (1): 5913. https://doi.org/10.1038/s41598-017-06174-3.

# Notes

# Notes

## Notice to purchaser

miND® is a registered trademark of TAmiRNA GmbH, Vienna, Austria.

RealSeq® is the Registered Trademark of RealSeq Biosciences, Inc. All other brands and names contained herein are the property of their respective owners.

A patent was filed for the invention related to novel spike-in oligonucleotides for absolute quantitation of nucleotide sequence data under WO2018138334A1.

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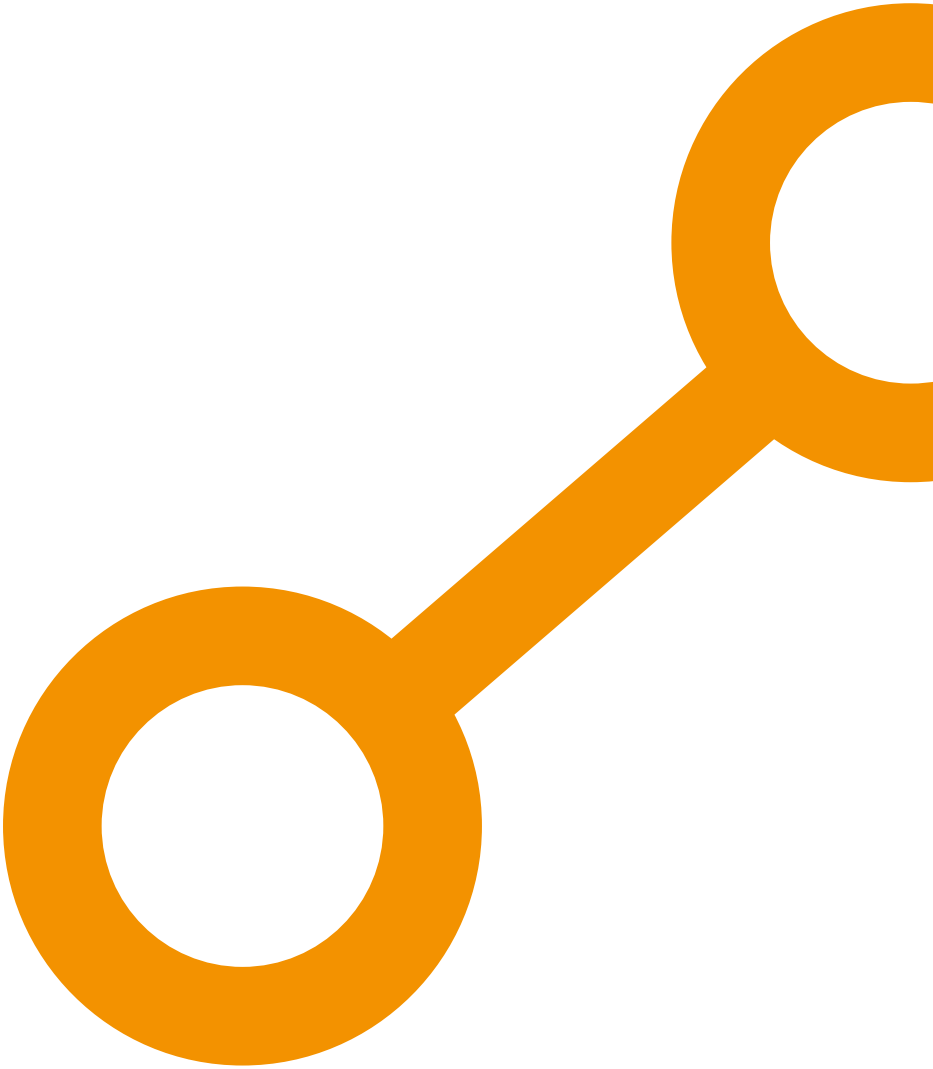

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