

**Catalog No. 11820-025 25 Reactions****Overview and Intended Use**

Marligen's *Vantage™* microRNA Labeling Kit is suitable for use in detecting microRNA expression levels and is compatible with Marligen's *Vantage™* Multiplex Detection Panels and other Luminex™ microRNA profiling assays.

MicroRNAs are a class of small molecules, about 21-23 nucleotides in length that regulate gene expression in a variety of manners including translational repression, mRNA cleavage, methylation, and deadenylation. Marligen's *Vantage™* microRNA Labeling Kit utilizes a proprietary labeling technology to provide exceptional sensitivity for the detection of endogenous microRNAs. This method can be used to label any RNA sample including total RNA, enriched low molecular weight (LMW) RNA, and degraded RNA. It is suitable for use with enriched RNA extracted from Formalin-Fixed Paraffin Embedded (FFPE) tissues.

Principle of Method

This simple protocol consists of just two steps. The 3' end of the RNA is poly(A) tailed followed by the ligation of biotinylated 3DNA™ DNA dendrimers containing 15 biotins to provide signal amplification.

Important Information**READ ENTIRE PROTOCOL BEFORE USE****ADDITIONAL PRECAUTIONS SHOULD BE TAKEN TO PREVENT THE DEGRADATION OF RNA.****Precautions to take when working with RNA:**

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

1. The RNA area should be located away from microbiological work stations
2. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
3. There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
4. All RNA solutions should be prepared using molecular biology grade nuclease-free water
5. Clean all surfaces with commercially available RNase decontamination solutions

6. When working with purified RNA samples, ensure that they remain on ice during downstream applications

Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents.

This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

If you have any questions concerning the use of this product, please contact Marligen Biosciences, Inc. at (866) 464 4990 or visit www.marligen.com.

Components included with this kit:

10X Reaction Buffer	37.5 µL
25mM MnCl ₂	37.5 µL
ATP Mix	25 µL
PAP Enzyme	25 µL
5X <i>Vantage™</i> Ligation-Biotin Mix	100 µL
T4 DNA Ligase	50 µL
Stop Solution	62.5 µL

Storage Conditions

Store all components at -20°C.

Handling Instructions:

1. Thaw the following components at room temperature: 10X Reaction Buffer, 25mM MnCl₂, 5X *Vantage™* Ligation-Biotin Mix, and Stop Solution. Once thawed, vortex, and spin down briefly prior to use.
2. Thaw the following components on ice: ATP Mix, PAP Enzyme and T4 DNA Ligase. Once thawed these components should be, spun down, and kept on ice at all times. **IMPORTANT: Do not vortex these components.**

Materials and Equipment Required But Not Supplied:

- Nuclease-free water (Ambion Cat. No. AM9932 or equivalent)
- 1M Tris pH 8.0 (Sigma Cat. No. T2694) diluted to 1mM Tris, pH 8.0.
- RNase inhibitor (Superase-In Ambion Cat. No. AM2694 or equivalent)
- Microcentrifuge
- Thermocycler or incubator set at 37°C (avoid water baths and incubators that allow condensation droplets to form on the insides of tubes)
- 1.5 mL nuclease-free microfuge tubes

Usage Notes for the Labeling Protocol

The *Vartage*[™] microRNA Labeling Kit can label many types of input RNA. RNA samples include total RNA, RNA extracted from FFPE tissues and enriched LMW RNA.

Some applications may require enrichment for optimal profiling. For example, to distinguish mature and precursor microRNAs, enrichment may be necessary. In addition, degraded total RNA samples should be enriched.

1. Table 1 outlines the recommended input RNA for labeling a particular sample type.

Table 1

RNA sample	Input for <i>Vartage</i> [™] Labeling
Total RNA (containing large and LMW RNAs)	0.5-4 µg (Note: maximum amount of input RNA is 4 µg)
Enriched LMW RNA	Enriched from 0.5-4 µg total RNA
FFPE RNA	100 ng

2. Labeling RNA Samples for use with the *Vartage*[™] Multiplex Detection Panels. It is recommended that 1-2 µg of labeled RNA is used per reaction. Less amounts of RNA may be used per reaction, but it is recommended that a pilot study is carried out to determine the optimal amount of starting RNA needed for a particular sample type. If duplicates are to be performed in the detection assay it is recommended that the user double the amount of input RNA to be labeled and split the sample accordingly at the detection step.

3. **IMPORTANT:** DNase will interfere with this labeling method. If the RNA sample being labeled has been treated with a DNase, we highly recommend that this enzyme is inactivated prior to labeling:

Inactivation of DNase:

1. Heat RNA sample at 95°C for 5 minutes.
2. Place sample on ice until ready to label.

Labeling Protocol

Part 1: Poly (A) Tailing

1. Adjust the volume of input RNA to 10 µL with nuclease-free water in a nuclease-free PCR tube.

Optional: Add manufacturer's recommended amount of RNase inhibitor (e.g. 0.5 µL Superase-In) prior to adding input RNA to nuclease-free PCR tube, add input RNA and adjust volume to 10 µL with nuclease-free water.

2. Dilute the **ATP Mix** in 1mM Tris (pH 8.0) according to the sample type in Table 2 below:

Table 2

RNA sample	Dilution of ATP Mix
Total RNA (containing large and LMW RNAs)	1:500
RNA samples extracted from FFPE	1:50
Enriched LMW Samples:	
quantitated	Use dilution factor: 5000 ÷ ng input LMW RNA
non-quantitated	Use dilution factor: 1000 ÷ µg input total RNA

3. Add the following to the tube containing 10 µL of total RNA. The final volume will be 15 µL

10X Reaction Buffer	1.5 µL
25mM MnCl₂	1.5 µL
diluted ATP Mix (diluted according to Step 2)	1 µL
PAP Enzyme	1 µL

4. Mix gently (**IMPORTANT: DO NOT VORTEX**) and spin down.

5. Incubate at 37°C in a thermocycler for 15 minutes. (Discard any unused, diluted **ATP Mix** from **Step 2**.) Place the tubes on ice immediately after the 15 minutes incubation.

Part 2: Ligation

1. Briefly spin down the 15 µL of Poly (A)-tailed RNA and place immediately on ice. (**IMPORTANT: Do not allow the contents of the tube to warm significantly.**)

2. Add 4 µL **5X *Vartage*[™] Ligation-Biotin Mix**.

3. Add 2 µL of **T4 DNA Ligase**.

4. Mix gently and spin down.

5. Incubate at room temperature for 30 minutes.

6. Stop the reaction by adding 2.5 µL **Stop Solution**. Mix and spin down the 23.5 µL of the ligated sample.

The samples are now ready for analysis

Technical Support

For further technical assistance please contact us at (866) 464 4990 ext 102 or by email at technical.support@marligen.com. Technical support and troubleshooting guides for these products can also be found on our website at www.marligen.com

Related Products

To see our full line of *Vartage*[™] microRNA analysis products visit our website at www.marligen.com

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