Catalog No. AM100044 **25 Reactions**

APPLICATION GUIDE

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Product Description

OriGene's Vantage™ microRNA Labeling Kit is suitable for use in detecting microRNA expression levels and is compatible with OriGene'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. OriGene'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, tailing and ligation. The 3'end of the RNA is poly(A)-tailed followed by the ligation of biotinylated 3DNA™ DNA dendrimers comprising 15 biotins to provide signal amplification.

PRECAUTIONS

Read entire protocol before use

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 OriGene Technologies at 1-888-267-4436 (301-340-3188 outside the US) or visit www.origene.com.

Components in the kit

10X Reaction Buffer	37.5 µL
25mM $MnCl_2$	37.5 µL
ATP Mix	25 µL
PAP Enzyme	25 µL
5X Vantage™ Ligation-Biotin Mix	100 µL
T4 DNA Ligase	50 µL
Stop Solution	62.5 µL

Store all kit components at -20ºC.

Handling Instructions

1. Thaw the following components at room temperature:
    - 10X Reaction Buffer
    - 25mM $MnCl_2$,
    - 5X Vantage™ Ligation-Biotin Mix
    - Stop Solution.

   Once thawed, vortex, and spin down briefly prior to use.

2. Thaw the following components on ice:
    - ATP Mix,
    - PAP Enzyme
    - 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.
- RNase inhibitor (Superase-In Ambion Cat. No. AM2694 or equivalent)
- Microcentrifuge
- Thermocycler or incubator set at 37ºC (avoid water baths and incubators that allow condensation droplets to form on the insides of tubes)
- 1.5 mL nuclease-free microcentrifuge tubes

RNA Sample Requirements

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

Total RNA is the preferred samples and will usually give optimal results. Some applications, however, 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.

For use with Vantage™ microRNA Detection Panels, it is recommended that 0.5-2 µg of labeled total RNA is used per labeling reaction. Smaller 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. Table 1 outlines the recommended input RNA for labeling a particular sample type.

Table 1. Recommended input RNA amounts

RNA sample	Input for *Van*tage™ Labeling
Total RNA (containing large and LMW RNAs)	0.5-4 µg (maximum amount of input RNA is 4 µg)
Enriched LMW RNA	Enriched from 0.5-4 µg total RNA
FFPE RNA	100 ng

IMPORTANT: Residual DNase will reduce with the labeling efficiency. If the RNA sample being labeled has been treated with a DNase, we highly recommend heat inactivation prior to labeling:

Inactivation of DNase

a. Heat RNA sample at 95°C for 5 minutes.

b. Place sample on ice until ready to label.

Related Products

Catalog Number

Vantage™ Total RNA Purification Kit NP100026

Vantage™ microRNA Purification Kit NP100028

Vantage™ microRNA Detection Kit (50 reactions) AM100091

Vantage™ microRNA Detection Kit (200 reactions) AM100092

Vantage™ microRNA Oncolology Detection Panel AM100045

Vantage™ microRNA Pancreatic Cancer Detection Panel AM100046

Vantage™ microRNA Breast Cancer Detection Panel AM100047

Vantage™ microRNA Ovarian Cancer Detection Panel AM100048

Vantage™ microRNA Cardiac Detection Panel AM100049

Vantage™ microRNA Diabetes Detection Panel AM100050

Vantage™ microRNA Hypoxia Detection Panel AM100051

Vantage™ microRNA Prostate Cancer Detection Panel AM100052

Vantage™ miR-Plex Control AM100090

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

Table 2. Dilution of ATP Mix

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 LMW RNA	Use dilution factor: 5000 ÷ ng input LMW RNA
Non-quantitated LMW RNA	Use dilution factor: 1000 ÷ µg input total RNA

4. 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
25 mM MnCl2	1.5 µl
Diluted **ATP Mix** diluted according to Table 2)	1 µl
PAP enzyme	1 µl

5. Mix gently (IMPORTANT: DO NOT VORTEX) and spin down.
6. Incubate at 37ºC 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 Vantage™ 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.

Frequently Asked Questions (FAQs)

How should I isolate and purify my RNA before using the Vantage™ MicroRNA Labeling kit?

OriGene highly recommends the use of the Vantage™ Total RNA Purification Kit (NP100026). However, RNA purified with kits from Qiagen, Ambion, and Life Technologies has been used successfully. After purifying your RNA, we recommend that you check your RNA sample for degradation and genomic DNA contamination on an agarose gel or an Agilent Bioanalyzer™.

Should I treat my RNA sample with DNase?

While treating an RNA sample with DNase is not required for Vantage™ microRNA labeling, it is a good practice since your RNA concentration will be more accurate. Prior to treating your RNA sample with DNase, you should check your sample with a spectrophotometer. The OD 260/280 ratio should be between 1.9 and 2.1. You should also run your RNA sample on a gel or Agilent Bioanalyzer™ to check for genomic contamination and degradation of your sample. You should only treat your RNA sample with DNase if you have genomic DNA contamination. After treatment with DNAse, you must completely inactivate the DNase before making labeling, since DNase will destroy labeled microRNA. See protocol for recommended heat inactivation steps.

What is a 3DNA dendrimer?

A 3DNA dendrimer is a signal amplification molecule made from DNA. Each dendrimer has a “core” that consists of a matrix of double-stranded DNA, as well as an outer surface comprised of ten to hundreds of single-stranded arms. The 3DNA dendrimer used in the Vantage™ microRNA labeling kit has fifteen arms, each labeled with a single biotin molecule. For more information 3DNA dendrimer technology, see http://www.genisphere.com/about_3dna.html.