

Catalog No. AM100091

50 Reactions

Catalog No. AM100092

200 Reactions

APPLICATION GUIDE

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Overview and Intended Use

The Vantage™ microRNA Detection Kit offers researchers a fast and simple method for profiling the expression levels of multiple microRNAs from many different sample types including total RNA, enriched low molecular weight (LMW) RNA, and degraded RNA. The assays are configured for the xMAP® bead array allowing for the detection of multiple microRNAs in one sample. In addition, the 96-well format allows many samples to be analyzed in one run.

MicroRNAs are a class of small molecules, about 21-23 nucleotides in length that regulate gene expression by various methods including translational repression, mRNA cleavage, methylation, and deadenylation. Differences in the expression levels of microRNAs have been associated with the pathogenesis of many diseases, including cancer. By measuring the expression levels of microRNAs, researchers obtain a better understanding of the processes involved in tumor development and progression. In addition, researchers can observe distinct expression patterns associated with particular stages of disease.

Principle of Method

The Vantage™ microRNA Detection Kit utilizes a simple, hybridization procedure for detection of biotinylated microRNA on the Luminex® instrument. Samples containing microRNA are labeled with a biotin dendrimer (multiple biotins attached to one oligo) using the Vantage™ microRNA Labeling Kit (sold separately, cat.no. AM100044). The biotinylated samples are incubated with a Bead Mix containing a mixture of different fluorescently dyed xMAP® beads. Each distinct xMAP® bead is coupled with a unique probe that recognizes a specific microRNA. Probe-coupled bead or bead mixes for specific microRNAs are sold separately. The beads and sample are incubated at 60°C allowing the microRNAs present in the sample to hybridize to the specific probes. Following hybridization, the samples are subjected to a high stringency wash to remove any non-specific binding. Finally, the samples are incubated with streptavidin-phycoerythrin (SAPE), which binds to the biotinylated microRNA hybridized to the xMAP® bead. The samples are read on Luminex® or Luminex-based instruments (e.g. BioPlex®) that detect the specific microRNAs present in the sample by their unique bead region and quantify the microRNAs by the intensity of the SAPE signal.

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

Name	50 rxns	200 rxns
Hybridization Buffer	1.25 mL	5 mL
Detection Reagent	55 µL	220 µL
Wash Buffer	2 x 10 mL	4 x 10 mL
SAPE Diluent	25 mL	4 x 25 mL
Aluminum Plate Sealers	2 Each	8 Each
Filter Plate	1 Each	5 Each

Handling Instructions

The kit is shipped on ice packs. Upon receipt, the following components should be stored at 2-8°C:

Detection Reagent

Wash Buffer

SAPE Diluent

Plate Sealers and Filter Plate may be stored at room temperature.

Materials and Equipment Required But Not Supplied

- Vantage™ MicroRNA Catalog or Custom Panel (bead mix containing probes for specific microRNAs)
- Nuclease-free PCR stripwell plate or nuclease-free PCR tubes
- 1.5 mL RNase free microfuge tubes
- Barrier micropipette tips
- Nuclease-Free water (Ambion Cat. No. AM9934 or equivalent)
- Microcentrifuge
- Thermocycler or heating block set to 60°C
- Plate Shaker
- Vortex Mixer
- Sonicating waterbath
- 96-well filter plate vacuum manifold
- Luminex Instrument
- **Optional, but recommended:** RNase Inhibitor (Suprase-In, Ambion Cat. No. AM2694 or equivalent)

Related Products

	Catalog Number
Vantage™ Total RNA Purification Kit	NP100026
Vantage™ microRNA Purification Kit	NP100028
Vantage™ microRNA Labeling Kit	AM100044
Vantage™ microRNA Oncology 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

Assay controls

1. Control 1 on bead region 49 is included in most Vantage™ microRNA Bead Mixes. This probe detects 5.8S RNA that is ubiquitously expressed in mammalian cells and is selected as a house-keeping gene for the Vantage™ Detection Kits. A signal of 4000-20000 MFI is typically observed when using 1-2 µg of high quality (RIN >8) total RNA.
2. Additional assay controls are available from OriGene. The Vantage™ MiR-plex Control contains synthetic biotinylated RNAs including 5.8S control. There is no need to label this sample as the MiR-plex Control is already biotinylated. To assay the MiR-plex Control, add 20 µL of the Vantage™ miR-plex Control into 33 µL of the Hybridization/Bead Mix at Hybridization Step 6, then follow protocol as described.

Assay Set-up

1. Prepare biotin-labeled RNA using the Vantage™ microRNA Labeling Kit (Cat. No. AM100044).
2. Use 0.5-3 µg of labeled RNA per reaction. If duplicates are to be performed in the detection assay, double the amount of input RNA to be labeled and split the sample at the hybridization step.

Note: Less than 0.5 µg/reaction may be used for some samples. However, it is recommended that a pilot study is carried out to determine the optimal

amount of labeled RNA for a particular sample type. Refer to the protocol for Vantage™ microRNA Labeling Kit for further details on sample labeling.

3. Set thermocycler or heating block to 60°C.
4. Warm up the Luminex or Luminex-based instrument.
5. If desired, incubate wash buffer at 60°C. Increased assay specificity may be achieved by increasing temperature of wash buffer to 60°C. However, increasing the wash temperature may reduce overall assay signal.

Luminex Instrument Setup

Set up the instrument as described in the user's manual. Setup details specific to this kit are described below:

1. The XY platform heater should be off.
2. Set the events/bead to 50.
3. Set the minimum events to 20.
4. Enter the number of samples.
5. Set the sample size to 50 µL.
6. Set the flow rate to Fast.
7. Enter the bead region numbers as indicated in the Bead Mix insert.
8. Check the probe height and adjust it, if necessary, to accommodate the filter plate.
9. Perform 1 prime with sheath fluid, 1 alcohol flush, and 2 sheath fluid washes.

10. Adjust Luminex Instrument to High Gain Setting

A high gain setting for the Luminex instrument is recommended to provide the best results. Luminex instruments from different vendors may have different protocols for setting the instrument to high gain. Below are instructions using the Luminex IS 2.3™ or LDS 1.7 software with a Luminex 100 or 200 instrument. See manufacturer's guidelines for other instruments or software (e.g. BioPlex®).

- a) Create a new lot number for CAL2 and enter lot number with an HG at the end to designate High Gain.
- b) Record the CAL2 Calibrator target "RP1" which is usually around 3832.
- c) Multiply the CAL2 Calibrator target "RP1" by 4.55 to get a new target value of approximately 17,436.
- d) Enter the new Calibrator target "RP1" as the value for your New CAL2 lot.
- e) Run the CAL2 calibration.

Detection Protocol

Part I: Hybridization

During this step the microRNAs present in the sample are hybridized to their complimentary sequences on the xMAP® beads.

1. Vortex the Bead Mix vigorously for 20 seconds.
2. Sonicate the Bead Mix in a sonicating waterbath for 2 minutes.
3. Prepare the Hybridization/Bead Mix based on the number of reactions to be run in the assay as illustrated in Table 1.

Table 1. Preparation of Hybridization Bead Mix

Component	Volume per reaction	Volume per 25 reactions	Volume per 50 reactions
Hybridization Buffer	25 µL	625 µL	1250 µL
Bead Mix	8 µL	200 µL	400 µL

1. Vortex the Hybridization/Bead Mix to ensure that it is fully mixed.
2. Add 33 µL of the Hybridization/Bead Mix into each well of a nuclease-free PCR stripwell plate or into each nuclease-free PCR tube.
3. Transfer 20 µL of the labeled RNA sample (prepared using Vantage™ microRNA Labeling Kit) into the 33 µL of the Hybridization/Bead Mix in the PCR wells or tubes, mix by pipeting up and down.
4. **Note:** If duplicates are to be performed, add 10 µL of the labeled RNA sample to the 33 µL of the Hybridization/Bead Mix in the PCR wells or tubes, bring the volume to 53 µL by adding 10 µL nuclease-free water, and mix by pipeting up and down.
5. Hybridize the reactions at 60°C by using a thermocycler or heating block for 120 minutes with continuous shaking at 400 rpm. Protect the reactions from light during this incubation.
6. **Note:** If shaking is not possible during this step, the MFI signals may be slightly reduced. However the overall results will not be affected.

Part II: Washing and Detection

During this part of the protocol, non-specific binding is removed by subjecting the reactions to high stringency washes. The specific microRNAs present in the sample are then detected by labeling the biotins with streptavidin-phycoerythrin (SAPE).

Notes

- a. **IMPORTANT:** Do not allow the filter membrane to dry throughout the transfer, wash and detection steps.
- b. It is highly recommended to add 1 unit/μL of RNase Inhibitor to the SAPE Diluent.
- c. It is important to apply a gentle vacuum of ~2-4 in Hg during all wash steps. Higher vacuum may result in the loss of beads and reduced bead count.
- d. During all wash steps, cover unused wells with a plate sealer to ensure a seal necessary to pull a vacuum.

A. Reagent Preparation

1. Prepare SAPE Detection Reagent as shown in Table 2.
2. Mix the SAPE Detection Reagent by gentle vortexing.

Table 2. Preparation of Detection Reagent

Component	Volume per reaction	Volume per 25 reactions	Volume per 50 reactions
Detection reagent	1 μL	25 μL	50 μL
SAPE Diluent	100 μL	2.5 mL	5 mL
RNase Inhibitor*	100 units	2500 units	5000 units

*Optional, but recommended.

B. Washing

1. Cover unused wells with a plate sealer.
2. Pre-wet the wells in filter plate by adding 100 μL Wash Buffer to each well. Apply gentle vacuum to remove buffer. Blot plate on absorbent paper towels.
3. Transfer the hybridization reactions to the pre-wet wells. Apply gentle vacuum to remove buffer.
4. Remove vacuum and immediately add 100 μL of Wash Buffer to each well and apply gentle vacuum to remove buffer. Repeat this wash step for total of 3 washes.
5. Remove vacuum and add 100 μL SAPE Diluent to each well and apply gentle vacuum to remove diluent.
6. Remove plate from manifold. Blot the bottom of the filter plate dry on a clean paper towel.

C. Detection

1. Add 100 µL of SAPE Detection Reagent into the washed wells of the filter plate.
2. Incubate the filter plate in dark for 30 minutes at room temperature.
3. **Note:** To increase MFI signal, the plate may be shaken at 400 rpm during this incubation. Protect the reactions from light.
4. Add 100 µL of SAPE Diluent to each well and apply vacuum to remove buffer. Repeat this wash step for a total of 3 washes.
5. Blot the bottom of the filter plate dry on a clean paper towel.
6. Add 100 µL of SAPE Diluent into each well.
7. Resuspend the beads in the filter plate by shaking for 2 minutes at 400 rpm or by pipeting up and down.
8. Read the filter plate in Luminex instrument at high gain setting (see Luminex Instrument Set-up).

Protocol Summary

1. Vortex and sonicate Bead Mix (from Detection Panel)
↓
2. Prepare Hybridization Bead Mix (Table 1)
↓
3. Add 33 μ l of Hybridization Bead Mix to PCR tube or plate.
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4. Add 20 μ l of labeled RNA to the Hybridization Bead Mix
↓
5. Hybridize for 120 minutes @ 60°C.
↓
6. Prepare SAPE Detection Reagent (Table 2)
↓
7. Pre-wet filter plate, vacuum and blot
↓
8. Transfer hybridization reactions to filter plate, vacuum and blot
↓
9. Wash 3 times with 100 μ l of Wash Buffer, vacuum, and blot
↓
10. Wash one time with 100 μ l SAPE diluent, vacuum and blot
↓
11. Add 100 μ l of SAPE Detection Reagent to each well
↓
12. Incubate the filter plate in dark for 30 minutes with shaking @ 400 rpm
↓
13. Remove SAPE Detection Reagent by vacuum and blot
↓
14. Wash 3 times with 100 μ l of SAPE Diluent
↓
15. Blot and dry bottom of filter plate
↓
16. Add 100 μ l of SAPE Diluent into each well
↓
17. Shake for 2 minutes @ 400 rpm
↓
18. Read the filter plate in the Luminex instrument on the high gain setting