

**Catalog No. 11831-050**

**50 Reactions**

### 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 on 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.

The specific microRNAs detected in the Vantage™ Oncology Detection Panel 1 are given in Appendix A. These microRNAs have been associated with a variety of solid tumors (ref.1-5.)

### Principle of Method

The Vantage™ microRNA Detection Kit utilizes a simple, hybridization procedure where samples are ready for detection on the Luminex® reader within 90 minutes. The samples are first labeled with multiple biotins using the Vantage™ microRNA Labeling Kit (Kit sold separately. See Cat. No. 11820-025). 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. 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.

### Terms and Conditions

By opening this Assay Product (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or

using this Assay Product in any manner, you are consenting to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Assay Product for a full refund prior to using it in any manner. You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Assay Product or any portion of this Assay Product, including without limitation the microsphere beads contained herein, only with Luminex Corporation's laser based fluorescent under the name Luminex Instrument.

### 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](http://www.marligen.com).

### Components included with this kit:

Component	Amount
Hybridization Buffer	1.25 mL
Oncology Panel 1 Bead Mix	400 µL
Detection Reagent	55 µL
Wash Buffer	2 x 10 mL
SAPE Diluent	25 mL
Aluminum Plate Sealers	2 Each
Filter Plate	1 Each

### Storage Conditions:

Store all components at 2-8°C.

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

## Materials and Equipment Required But Not Supplied:

Nuclease-free PCR stripwell plate or nuclease-free PCR tubes  
1.5 mL RNase free microfuge tubes  
Plugged micropipette tips  
Nuclease-Free water (Ambion Cat. No. AM9934 or equivalent)  
Microcentrifuge

Thermocycler or heating block at 60°C

Plate Shaker

Vortex Mixer

Sonicated waterbath

96-well filter plate vacuum manifold

Luminex Instrument

**Optional:** RNase Inhibitor (Suprase-In, Ambion Cat. No. AM2694 or equivalent)

## Important Information

### READ ENTIRE PROTOCOL BEFORE USE

### ADDITIONAL PRECAUTIONS SHOULD BE TAKEN TO PREVENT THE DEGRADATION OF 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 at least 0.05% DEPC-treated autoclaved water or 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.

## Assay controls

1. Control 1 (bead 49) detects 5.8S RNA that is ubiquitously expressed in mammalian cells and is selected as a house-keeping gene for the *Vartage*<sup>TM</sup> Detection Kits. A signal of 4000-10000 MFI is typically observed when using 1-2 µg of high quality (RIN >8) total RNA.
2. An additional assay control is available from Marligen. The *Vartage*<sup>TM</sup> MiR-plex Control (Cat. No. 11830-001) contains 7 synthetic biotinylated RNAs including 5.8S, miR-21, and miR-9 that are detected by the *Vartage*<sup>TM</sup> Oncology Detection Panel 1.

Add 20 µL of the *Vartage*<sup>TM</sup> miR-plex Control into 33 µL of the Hybridization/Bead Mix at **Hybridization Step 6**, then follow protocol as described. Expected results for this assay control is shown in Appendix B.

**Note:** There is no need to label this sample as the MiR-plex Control is already biotinylated.

## Set-up Prior to Starting Detection Protocol

1. Prepare labeled RNA. Prior to using this detection kit the microRNAs present in the samples must be labeled with biotin. To obtain optimal results, it is recommended that the *Vartage*<sup>TM</sup> microRNA Labeling Kit (Cat. No. 11820-025) is used to label samples.
2. Use 0.5-2 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 accordingly.

**Notes:** 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 *Vartage*<sup>TM</sup> 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.

## Luminex Instrument Setup

### A. 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 table in Appendix A.
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.

### B. Adjust Luminex Instrument to High Gain Setting

A high gain setting for the Luminex instrument is recommended to provide the best results. Each specific software used with the Luminex or Luminex-based instrument may have different instructions for obtaining the high gain setting. Below are instructions using the Luminex 2.3<sup>TM</sup> software. Please see manufacturer's guidelines for instrument/software specific instructions (e.g. BioPlex®).

1. Create a new lot number for CAL2 and enter lot number with an HG at the end to designate High Gain.

- Record the CAL2 Calibrator target "RP1" which is usually around 3832.
- Multiply the CAL2 Calibrator target "RP1" by 4.55 to get a new target value of approximately 17,436.
- Enter the new Calibrator target "RP1" as the value for your New CAL2 lot.
- 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.**

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

**Table 1 Hybridization/Bead Mix Preparation**

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

- Vortex the **Hybridization/Bead Mix** to ensure that it is fully mixed.
- 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.
- Transfer 20 µL of the labeled RNA sample (prepared using *Vartage*<sup>TM</sup> microRNA Labeling Kit) into the 33 µL of the **Hybridization/Bead Mix** in the PCR wells or tubes, mix by pipeting up and down.

**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.

**IMPORTANT:** As noted in the Set-up, duplicates should have double the amount of input RNA labeled with the *Vartage*<sup>TM</sup> microRNA Labeling Kit.

- Hybridize the reactions at 60°C by using a thermocycler or heating block for one hour with continuous shaking at 400 rpm. Protect the reactions from light during this incubation.

**[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: Detection

**During this step, 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 SAPE.**

#### Usage Notes

- IMPORTANT:** Do not allow the filter membrane to dry throughout the transfer, wash and detection steps.
- It is highly recommended to add 1 unit/µL of RNase Inhibitor to the **SAPE Diluent**.
- It is important to apply a slight vacuum of ~2-3 in Hg during all wash steps. Higher vacuum may result in the loss of beads and reduce bead count.
- During all wash steps, cover unused wells with a plate sealer to ensure a seal necessary to pull a vacuum.

#### A. Washes

- Pre-wet the wells in filter plate with 100 µL **Wash Buffer**.
- Transfer the hybridized reactions to each pre-wet well, cover unused wells with a plate and apply vacuum to evacuate.
- Remove vacuum and immediately add 100 µL of **Wash Buffer** to each well and apply vacuum to remove buffer. Repeat this wash step for total of 3 washes.

**Optional:** For customer convenience, all washes are performed at room temperature. As shown in Appendix B, increased assay specificity may be achieved by increasing temperature of wash buffer to 60°C. However, please note that increasing the wash temperature may reduce overall assay signal.

- Remove vacuum and add 100 µL SAPE Diluent to each well and apply vacuum to remove diluent. Remove plate from manifold.
- Blot the bottom of the filter plate dry on a clean paper towel.

#### B. SAPE Detection

- Prepare **SAPE Detection Reagent** as shown in Table 2.

**Table 2 SAPE Detection Reagent Preparation**

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

**Optional:** Add 1 unit of RNase Inhibitor (e.g. Superase-In, Ambion Cat. No. AM2694 or equivalent) per microliter of **SAPE Diluent**.

- Mix the **SAPE Detection Reagent** by vortexing.
- Add 100 µL of **SAPE Detection Reagent** into the washed well of the filter plate.
- Incubate the filter plate in dark for 30 minutes at room temperature.

**[Note:** To increase MFI signal, the plate may be shaken at 400 rpm during this incubation. Protect the reactions from light.]

- Add 100 µL of **SAPE Diluent** to each well and apply vacuum to remove buffer. Repeat this wash step for total of 3 washes.
- Blot the bottom of the filter plate dry on a clean paper towel.
- Add 100 µL of **SAPE Diluent** into each well to resuspend the beads in the filter plate by shaking for 2 minutes at 400 rpm or by pipeting up and down.
- Read the filter plate in Luminex instrument at high gain setting (see Luminex Instrument Set-up).

#### DATA Analysis

- Use the MFI data output from the Luminex Instrument to collect the raw MFI.
- Subtract the background MFI in negative control from the Sample MFI. Note: If after subtraction of background MFI signal is negative, regard results as zero i.e., that specific microRNA is not detectable in the sample.
- Control 1 (bead 49) detects 5.8S RNA that is ubiquitously expressed in mammalian cells and is selected as a house-keeping microRNA gene for the *Vartage*<sup>TM</sup> Oncology Panel 1. When comparing two samples, normalize the MFI with Control 1.

$$\text{Normalization factor} = \frac{\text{MFI of Control 1 in Control Sample}}{\text{MFI of Control 1 in Tumor or Treated Sample}}$$

#### For example:

Control 1 in normal tissue: MFI = 20000

Control 1 in tumor tissue: MFI = 16000

$$\text{Normalization factor} = \frac{20000}{16000} = 1.25$$

- Then multiply Tumor or Treated Sample MFI by the normalization factor 1.25.
- The fold change in microRNA expression can be calculated by dividing the normalized MFI for the Tumor or Treated Sample by the MFI for the Control Sample. Plot results using Excel or equivalent. Note that if an MFI value is zero (or close to 0 {<10}), the microRNA is not detectable in the sample and should be considered as not expressed.
- To determine assay precision, calculate standard deviation (SD) and assay coefficient of variation (CV). [%CV =SD/mean x 100%]. Assay CVs are typically less than 5% for technical replicates.

#### Technical Support

For further technical assistance please contact us at (866) 464.4990.ext.102 or [technical.support@marligen.com](mailto:technical.support@marligen.com).

Technical support and troubleshooting guides for these products can also be found on our website at [www.marligen.com](http://www.marligen.com).

#### Related Products:

To see our full line of *Vartage*<sup>TM</sup> microRNA analysis products visit our website at [www.marligen.com](http://www.marligen.com).

#### Limited Use License and Use Restrictions

A limited, research use only, license is conveyed to the purchaser of this product.

#### Trademarks

*Vartage*<sup>TM</sup> is a trademark of Marligen Biosciences, Inc. Luminex® and xMAP® are trademarks of Luminex Corporation.

#### References

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**APPENDIX A: TABLE OF microRNAs AND xMAP® BEAD REGIONS**

The sequences and nomenclature of the mature microRNAs are extracted from The miRBase Sequence Database version 12.0, released in September 2008, in Sanger Institute in UK. Names annotated with \* indicate a mature microRNA sequence that originated from a stem-loop molecule that generated two mature microRNA sequences. In these cases, one mature sequence has a standard name while the other sequence from the same stem-loop has an annotated name(\*). The nomenclature of the sequences detected by Vantage™ Oncology Detection Panel 1 is designated using the human sequence nomenclature. The equivalent mouse and rat sequences are indicated in the table below.

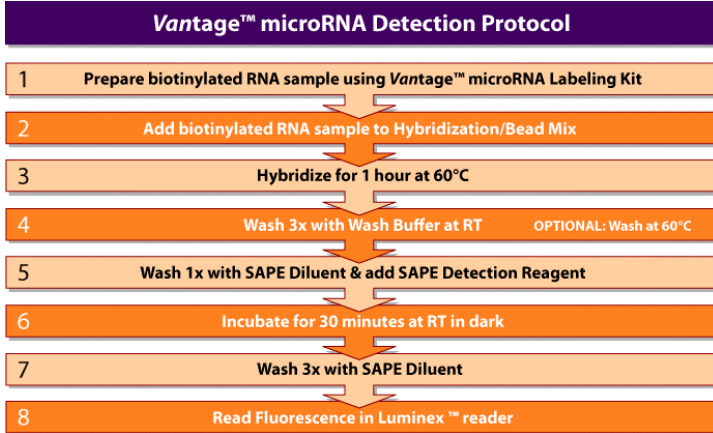
xMAP® Bead Number	Human microRNA Nomenclature	Human microRNA mature sequences	Equivalent Mouse (Mus musculus) Nomenclature	Equivalent Rat (Rattus norvegicus) Nomenclature	Notes
1	hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	mmu-let-7a	rno-let-7a	
2	hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUU	mmu-let-7c	rno-let-7c	
3	hsa-let-7g	UGAGGUAGUAGUUUGUACAGUU	mmu-let-7g		
4	hsa-let-7i	UGAGGUAGUAGUUUGUCUGUU	mmu-let-7i	rno-let-7i	
5	hsa-miR-100	AACCCGUAGAUCGAACUUGUG	mmu-miR-100	rno-miR-100	
6	hsa-miR-106a	AAAAGUGCUUACAGUGCAGGUAG	mmu-miR-106a		
7	hsa-miR-10a	UACCCUGUAGAUCGAAUUUGUG	mmu-miR-10a	rno-miR-10a-5p	
8	hsa-miR-10b	UACCCUGUAGAACGAAUUUGUG	mmu-miR-10b	rno-miR-10b	
9	hsa-miR-125a-5p	UCCUGAGACCCUUAACCUUGUGA	mmu-miR-125a-5p	rno-miR-125a-5p	
10	hsa-miR-125b	UCCUGAGACCCUUAACCUUGUGA	mmu-miR-125b-5p	rno-miR-125b-5p	
11	Not in use				
12	Not in use				
13	hsa-miR-132*	ACCGUGGCUUUCGAUUGUUACU			
14	hsa-miR-135a	UAUGGCUUUUUUAUCCUAUGUGA	mmu-miR-135a	rno-miR-135a	
15	hsa-miR-136	ACUCCAUUUGUUUGAUGAUGGA	mmu-miR-136	rno-miR-136	
16	hsa-miR-138	AGCUGGUGUUGUGAAUCAGGCCG	mmu-miR-138	rno-miR-138	
17	hsa-miR-141*	CAUCUCCAGUACAGUGUUGGA	mmu-miR-141*		
18	hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG	mmu-miR-16	rno-miR-16	
19	hsa-miR-17	CAAAGUGCUUACAGUGCAGGUAG	mmu-miR-17	rno-miR-17	
20	hsa-miR-181b	AACAUCAUUGCUGUCGGUGGGU	mmu-miR-181b	rno-miR-181b	
21	hsa-miR-185	UGGAGAGAAAGGCAGUUCUGA	mmu-miR-185	rno-miR-185	
22	hsa-miR-195	UAGCAGCACAGAAAUAUUGGC	mmu-miR-195	rno-miR-195	

<b>xMAP® Bead Number</b>	<b>Human microRNA Nomenclature</b>	<b>Human microRNA mature sequences</b>	<b>Equivalent Mouse (Mus musculus) Nomenclature</b>	<b>Equivalent Rat (Rattus norvegicus) Nomenclature</b>	<b>Notes</b>
23	hsa-miR-199a-5p	CCCAGUGUUCAGACUACCUGUUC	mmu-miR-199a-5p	rno-miR-199a-5p	
24	hsa-miR-200b*	CAUCUACUGGGCAGCAUUGGA	mmu-miR-200b*		
25	hsa-miR-205	UCCUUCAUUCCACGGAGUCUG	mmu-miR-205	rno-miR-205	
26	hsa-miR-20a	UAAAGUGCUUUAUGUGCAGGUAG	mmu-miR-20a	rno-miR-20a	
27	hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA	mmu-miR-21	rno-miR-21	
28	hsa-miR-210	CUGUGCGUGUGACAGCGGCUGA	mmu-miR-210	rno-miR-210	
29	hsa-miR-212	UACAGUCUCCAGUCACGGCC	mmu-miR-212	rno-miR-212	
30	hsa-miR-218	UUGUGCUUGAUCUAACCAUGU	mmu-miR-218	rno-miR-218	
31	hsa-miR-23b*	UGGGUUCUGGCAUGCUGAUUU			
32	hsa-miR-24-2*	UGCCUACUGAGCUGAAACACAG	mmu-miR-24-2*	rno-miR-24-2*	
33	hsa-miR-27a*	AGGGCUUAGCUGCUUGUGAGCA	mmu-miR-27a*	rno-miR-27a*	
34	hsa-miR-29a*	ACUGAUUUUUUUGGUGUUCAG	mmu-miR-29a*	rno-miR-29a*	
35	hsa-miR-29b-2*	CUGGUUUCACAUGGUGGCUUAG		rno-miR-29b-2*	
36	hsa-miR-29c*	UGACCGAUUUCUCCUGGUGUUC	mmu-miR-29c*	rno-miR-29c*	
37	hsa-miR-30d	UGUAAACAUCCTCCGACUGGAAG	mmu-miR-30d	rno-miR-30d	
38	hsa-miR-34a	UGGCAGUGUCUJAGCUGGUUGU	mmu-miR-34a	rno-miR-34a	
39	hsa-miR-34b*	UAGGCAGUGUCAUUAGCUGAUUG		rno-miR-34b	
40	hsa-miR-9	UCUUUGGUUAUCUAGCUGUAUGA	mmu-miR-9	rno-miR-9	
41	hsa-miR-93	CAAAGUGCUGUUCGUGCAGGUAG	mmu-miR-93	rno-miR-93	
42	hsa-miR-95	UUCAACGGGUUUUUUUGAGCA			
43	hsa-miR-96	UUUGGCACUAGCACAUUUUUGCU	mmu-miR-96	rno-miR-96	
44	hsa-miR-99a	AACCCGUAGAUCCGAUCUUGUG	mmu-miR-99a	rno-miR-99a	
45	hsa-miR-137	UUAUUGCUUAAGAAUACGCGUAG	mmu-miR-137	rno-miR-137	
46	hsa-miR-182*	UGGUUCUAGACUUGCCAACUA			
47	hsa-miR-221	AGCUACAUUGUCUGCUGGGUUUC	mmu-miR-221	rno-miR-221	
48	hsa-miR-372	AAAGUGCUGCGACAUUUGAGCGU			
49	NA	Control 1			

## APPENDIX B

### PERFORMANCE CHARACTERISTICS OF *VANTAGE*<sup>™</sup> ONCOLOGY DETECTION KITS

#### 1. Protocol Overview



#### 2. Assay Sensitivity

a. The limit of detection of the *Vantage*<sup>™</sup> microRNA Detection Kits for specific microRNAs was calculated using synthetic microRNAs labeled with the *Vantage*<sup>™</sup> microRNA Labeling Kit (Cat. No. 11820-025) and detected using Oncology Detection Panel 1 (Cat. No. 11831-050).

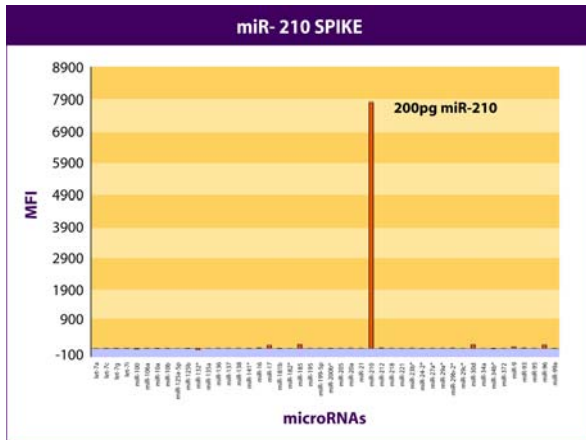
**Limit of Detection Range : 0.03-0.1 pg (5-15 attomoles)**

b. The limit of detection of the *Vantage*<sup>™</sup> microRNA Detection Kits in tissues was calculated using total RNA extracted from human brain labeled with the *Vantage*<sup>™</sup> microRNA Labeling Kit (Cat. No. 11820-025) and detected using Oncology Detection Panel 1 (Cat. No. 11831-050).

**Limit of Detection Range : 3-40 ng total RNA**

#### 3. Assay Specificity

a. No Cross-reactivity Observed Between Unrelated Sequences



200 pg of synthetic miR-210 labeled with the *Vantage*<sup>™</sup> microRNA Labeling Kit (Cat. No. 11820-025) and detected using Oncology Detection Panel 1 (Cat. No. 11831-050). MiR-210 lacks homology with any other sequence in the Oncology Detection Panel 1 and shows > 1% cross-reactivity with any other miR assay in this multiplex.

## b. Balance Between Specificity and Sensitivity for Closely Related Sequences

microRNA Site	Mature sequence	# nt different from miR-100	WASH TEMPERATURE					
			25° C		50° C		60° C	
			MFI	Cross-Reactivity	MFI	Cross-Reactivity	MFI	Cross-Reactivity
hsa-miR-100	AACCCGUAGAUCCGAACUUGUG	0	13057	100%	10992	100%	7743	100%
hsa-miR-99a	AACCCGUAGAUCCGAUCUUGUG	1	1082	8.3%	647	5.9%	100	1.3%
hsa-miR-10a	UACCCUGUAGAUCCGAUUUGUG	4	179	1.4%	3	<1%	2	<1%
hsa-miR-10b	UACCCUGUAGAACCGAAUUUGUG	5	29	<0.5%	2	<0.5%	3	<0.5%
hsa-miR-125b	UCCUGAGACCCUAACUUGUGA	6	1.3	<0.1%	0.4	<0.1%	0.1	<0.1%

400 attomoles of synthetic biotinylated miR-100 was incubated with the *Vartage*<sup>TM</sup> Oncology Detection Panel 1 (Cat. No. 11831-050). The assay was performed using the protocol as described in the package insert except different temperatures were employed at the wash step. Results show <10% cross reactivity with very similar sequences under standard protocol conditions (25°C). Negligible cross reactivity was observed for all of the other miR sites. Specificity for miR-100 increased by raising the wash temperature but the overall assay signal is reduced. In this assay, high sensitivity and low cross reactivity are maintained following the standard protocol, although greater specificity can be obtained by increasing wash temperature.

microRNA Site	Mature sequences	# nt different from let-7a	WASH TEMPERATURE			
			25° C		60° C	
			MFI	Cross-Reactivity	MFI	Cross-Reactivity
hsa-let-7a	UGAGGUAGUAGGUUGUAUAGUU	0	10769	100%	6789	100%
hsa-let-7c	UGAGGUAGUAGGUUGUAGGUU	1	4437	41%	1262	19%
hsa-let-7g	UGAGGUAGUAGUUUGUACAGUU	2	226	2.1%	0	0.0%
hsa-let-7i	UGAGGUAGUAGUUUGUGCUGUU	4	117	1.1%	1	0.0%

400 attomoles of synthetic let-7a was incubated with the *Vartage*<sup>TM</sup> Oncology Detection Panel 1 (Cat. No. 11831-050). The assay was performed using the protocol described in the package insert, except the wash step was also carried out at 60°C. With the exception of the very similar let-7c sequence, all miR sites in the assay show negligible cross-reactivity under standard protocol conditions. Specificity for let-7a increases significantly by increasing the wash temperature, although this decreases the overall assay signal. For the most sensitive assay, follow the standard protocol, but for higher specificity, increase the wash temperature.

## 4. *Vartage*<sup>TM</sup> MiR-plex Control Expected Results

<i>Vartage</i> <sup>TM</sup> microRNA Detection Kit	Oncology Detection Panel 1 (47-plex)	Breast Cancer Panel 1 (8-plex)	Pancreatic Cancer Panel 1 (12-plex)	Ovarian Cancer Panel (8-plex)	Cardiac Panel 1 (10-plex)	Diabetes Panel 1 (11-plex)	Hypoxia Panel 1 (10-plex)
Cat. No.	11831-050	11833-050	11832-050	11834-050	11835-050	11836-050	11837-050
microRNA	Expected MFI Values						
miR-1	-	-	-	-	Low	Low	-
miR-107	-	-	Low	-	-	-	Low
miR-126	-	Low	-	-	-	-	-
miR-203	-	-	-	Low	-	-	-
miR-21	High	High	High	High	High	-	High
miR-9	High	-	-	-	-	High	-
5.8S	Medium	Medium	Medium	Medium	Medium	Medium	Medium