

# Application Note

*Featuring BiOptix'  
Surface Plasmon Enhanced (SPE)  
Interferometry Technology for  
highly sensitive multiplexed biodetection*

Label free and Multiplex Detection of MicroRNAs with the BiOptix ACCOLADE™ Instrument

Mary S. Rosendahl, Ph.D.  
Director of Biochemistry

## Introduction

MicroRNAs (miRNAs) have gained considerable attention as potentially predictive biomarkers for a number of disorders. Mounting evidence suggests that miRNAs are differentially expressed in a variety of cancers and thus have potential for early detection, prognosis, and monitoring of response to treatment (Calin et al. 2006). Because of their small size and low concentrations, miRNAs in biological fluids are demanding targets for detection, requiring advanced diagnostic technologies. This application will describe protocols for the specific detection of cancer related miRNAs in the presence of a total RNA extract using the BiOptix ACCOLADE™ Surface Plasmon Enhanced common path interferometry (SPE) instrument. SPE differs from standard Surface Plasmon Resonance (SPR) detection methods, such as Biacore, in that it combines the high sensitivity of SPR with the higher stability and lower noise of a single-beam path for both reference and sample channels. The ACCOLADE™ can also simultaneously monitor multiple analytes in a single sample. In this application note, protocols are described that allow for the detection of picomolar concentrations of miRNAs by utilizing the ACCOLADE™ in combination with a signal enhancing monoclonal antibody that recognizes DNA-RNA hybrid duplexes (Hu et al., 2006).



## Materials & Methods

### Reagents

Oligonucleotides and synthetic microRNAs were obtained from Integrated DNA Technologies. Biotinylated anti-sense DNA oligos (sequences shown below) were used on the SensorChip surface as probes for specific target miRNA detection.

Channel 1 Biot-AS miD-21 5'- /5BiosG/TC TGA TCA ACA TCA GTC TGA TAA GCT A -3'

Channel 2 Biot-AS miD-24 5'- /5BiosG/TC TGA CTG TTC CTG CTG AAC TGA GCC A -3'

Channel 3 Biot-AS miD-205 5'- /5BiosG/TC TGA CAG ACT CCG GTG GAA TGA AGG A -3'

Channel 4 Biot-AS miD-221 5'- /5BiosG/TC TCT GAA ACC CAG CAG ACA ATG TAG CT -3'

FirstChoice™ Human Liver Total RNA was purchased from Ambion/Applied Biosystems (AM7960). NeutrAvidin SensorChips (Part No. C-40403) and ACCOLADE™ Instrument are products available from BiOptix Diagnostics, Boulder, Colorado. The S9.6 mouse monoclonal antibody was generously provided by Dr. S. Leppla (NIH). S9.6 is also available as a hybridoma cell line from ATCC (HB-8730). All other reagents were obtained from Sigma-Aldrich.

### Immobilization of DNA Probes

The NeutrAvidin SensorChip was first soaked in PBS (phosphate buffered saline) for 20-30 minutes to remove the preservative. Next the chip was conditioned by pipetting 1 ml of 0.05% SDS in 20 mM Tris, pH 7.4 over the active surface followed by 2 ml of PBS. The SensorChip was loaded and locked into the BiOptix Chip Charger as described in the ACCOLADE™ manual. The individual biotinylated oligos (Biot-AS miD-24-1, Biot-AS miD-155, Biot-AS miD-205 and Biot-AS miD-221, 100 nM in PBS) were loaded onto channels 1-4, respectively. The immobilization was allowed to proceed for 45 minutes with a second injection being performed at 20 minutes to maximize surface saturation. At the end of the loading, each channel was rinsed with 2 ml of PBS before removing the SensorChip from the charger. The chip was stored in PBS at room temperature.

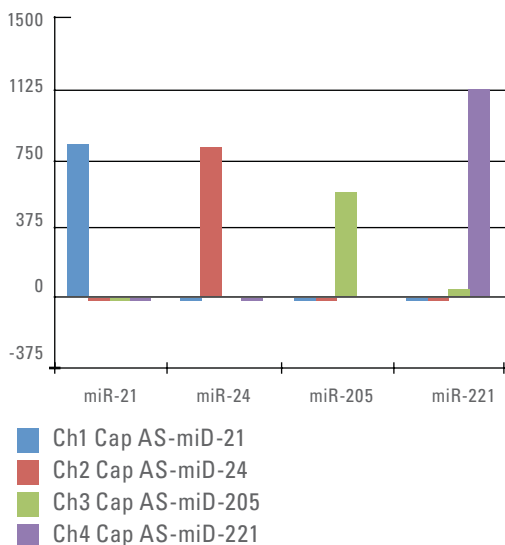
### General Assay Protocol

After loading the charged SensorChip into the ACCOLADE™, the RNase free running buffer (10 mM HEPES, 150 mM NaCl, 15 mM MgCl<sub>2</sub> (HBS-Mg) was introduced until the baseline stabilized (~30 min). Then, various solutions of miRNA analogs (diluted in running buffer) were injected at a flow rate of 60 µl/min for 20 minutes followed by a 5 minutes dissociation phase with running buffer only. At the end of each measurement the sensor surface was regenerated by a 20 seconds exposure to 50 mM NaOH.

## Experiments & Results

### Specific Detection of miRNAs using the ACCOLADE™

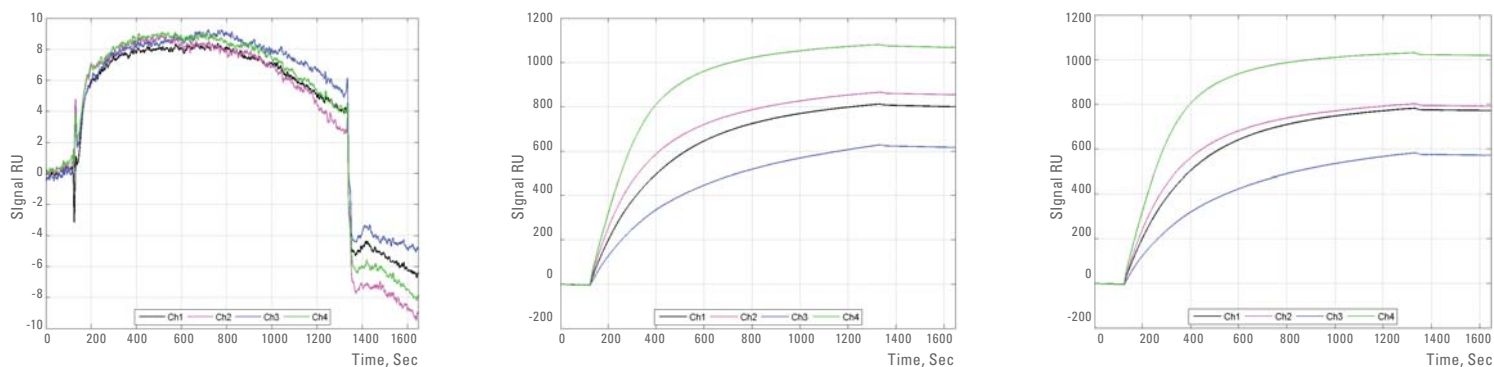
The SensorChip previously charged with the four anti-sense DNA probes was loaded into the ACCOLADE™ Instrument. Four separate samples of miRNA analogs (miR-21, 24, 205 and 221) were serially injected into the instrument to determine the specificity of the probe oligos. Only the individual channel that contained the complementary oligonucleotide sequence showed a response (Figure 1). Minimal cross reactivity for the non-complementary oligos was observed with the various miRNAs.



**Figure 1.** Specific Detection of four miRNAs. Individual channel responses were calculated by the BiOptix software. Variations in the total responses for complementary oligos are likely due to the different hybridization rates for duplex formation and their respective melting temperatures.

### Multiplex Detection of miRNAs in total RNA extract

Using a SensorChip prepared as described above, samples of 1 µg/ml total RNA extract from liver, a mixture of miRNA analogs (21, 24, 205 and 221) at 100 nM each, and the same mixture spiked with 1 mg/ml total RNA extract, were separately injected over the SensorChip and the ACCOLADE™ responses measured. The total RNA extract alone showed a minimal background effect of ~10 RU and did not significantly interfere with the multiplex detection of a mixture of miRNA analogs (Figure 2).



**Figure 2.** Sensorgrams for the analysis of a total RNA extract and a mixture of miRNAs spiked with total RNA. Panel A is the total RNA extract. Panel B is the miRNA mixture in running buffer (control). Panel C is the miRNA mixture spiked with total RNA extract. Graphs were taken from BiOptix Experiment Report.

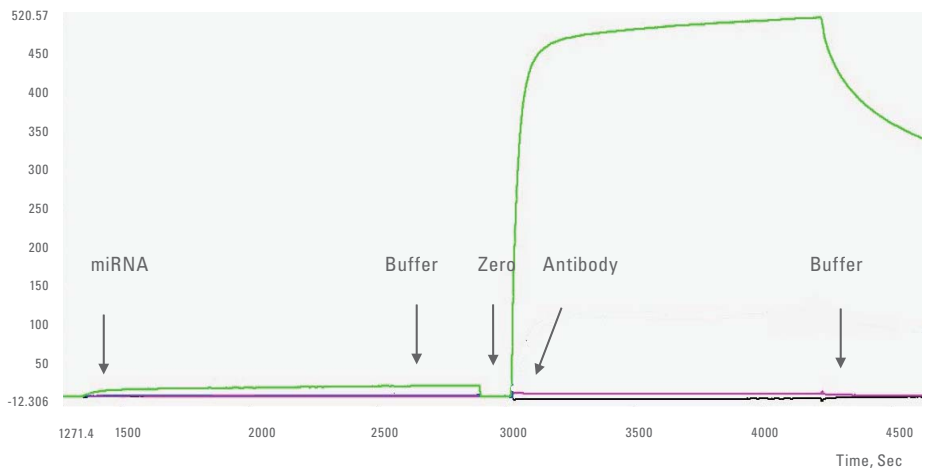
## Signal Amplification for Low Abundance miRNA Detection

Since miRNA are small and present in biological samples at picomolar concentrations, an ultra-sensitive protocol was developed for specific detection at physiologically relevant levels. This assay amplifies signal on the ACCOLADE™ with the mouse monoclonal antibody S9.6 that binds DNA-RNA duplexes without sequence specificity.

A SensorChip functionalized with NeutrAvidin was prepared by attaching a biotinylated oligo (B-AS-miD-221) onto one of the four channels using the BiOptix Chip Charger. A reference channel was prepared with B-AS-miD-24 as a control. The chip was loaded into the ACCOLADE™ instrument equilibrated in HBS-Mg. A solution containing 300 pM miRNA-221 in running buffer was injected for 20 minutes. The SensorChip was then flushed with buffer for 5 minutes followed by a 20 minutes injection of the S9.6 antibody at 2 µg/ml. To compensate for bulk effects and non-specific binding, the response on the reference channel was subtracted from the sensing channel. As seen in Figure 3, minimal response was observed with the reference channel while the channel with the DNA-RNA duplex showed a response of around 500 RU for a 300 pM solution of miRNA.

**Figure 3. Screen shot of miRNA signal amplification using the S9.6 antibody.**

The ACCOLADE™'s response for 300 pM miRNA-221 solution (20 min load, 60 µL/min) was ~15 RU versus ~500 RU after antibody signal enhancement (green). The reference channel with a non-complementary capture oligo showed essentially no response to miRNA and slight non-specific binding with the antibody. (<10 RU) (pink)



## Conclusion

In this report, we demonstrate multiplex detection of picomolar levels of cancer-related miRNAs using BiOptix' ACCOLADE™ instrument. This sensitive, rapid, label-free approach is a simple and inexpensive alternative to methods such as Northern blotting and qPCR that are currently being used to measure miRNA levels in biological samples.

## References

- Calin G and Croce CM (2006) *Nat Rev Cancer* 6:857-866. *MicroRNA signatures in human cancers.*
- Hu Z, Zhang A, Storz G, Gottesman S and Leppla SH (2006) *Nucleic Acids Research* 34:e52-59. *An antibody-based microarray assay for small RNA detection.*

## BiOptix Diagnostics Inc

1775 38th St., Boulder, CO 80301  
 General e-mail: [bioptix@bioptix.com](mailto:bioptix@bioptix.com)  
 Sales e-mail: [sales@bioptix.com](mailto:sales@bioptix.com)  
[www.BiOptix.com](http://www.BiOptix.com)  
 Telephone: 303.545.5550  
 Fax: 303.545.5551