

**The Advantages of  
NanoBioSensor™  
Detection/Measurement  
over ELISA, Lateral Flow, RT-  
PCR and other  
Macromolecule Assays.**

*Vista Therapeutics Products, Services  
and Pricing. January 2017*



The  
Awesome  
Power of  
Small

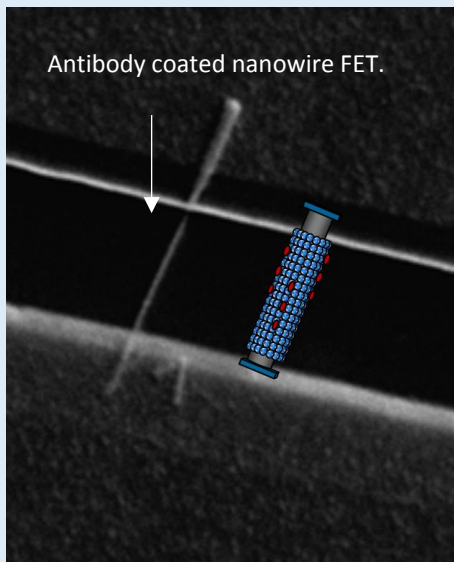
# Advantages of NanoBioSensor Detection/Measurement over ELISA, Lateral Flow, RT-PCR and other Macromolecule Assays.



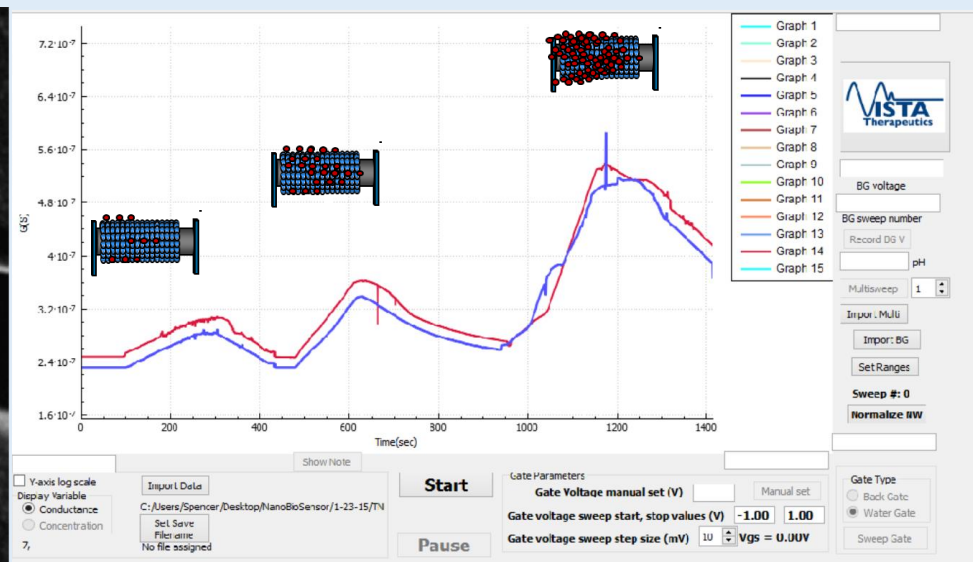
## Brief Summary of Vista's Nanowire-Scale Field Effect Transistor Assay for Biological Macromolecules. Most Macro-

molecules (proteins, RNA and DNA) useful as biomarkers are soluble in bodily fluids because they carry formal electric charges at physiological pH and thus generate electric fields. Field Effect Transistors (FETs) are transistors that control the flow of current through a circuit dependent upon the strength of the electric field that impinges of the transistor surface. At the nanoscale, FETs can be turned on or off by the binding of charged 'target' macromolecules to nanowire (NW) transistors. NW FETs can detect single viral particles. They can also detect most soluble proteins at the femtomolar level or lower. The conductivity through a given FET nanowire circuit is directly proportional to the number of charged molecules bound to their surface at any moment, which is a direct reflection of the concentration of the target molecule at that time. To provide specificity,

the nanowires are coated with 'capture' molecules specific to the 'target'. Vista has created a NanoBioSensor™ System that employs nanowire transistor-gated circuits coated with specific capture molecules that bind target molecules on a label-free, real-time continuous basis. This process is summarized in the figures to the right. Some of the advantages of NW-FET based bio-sensing over ELISA, Lateral flow and RT-PCR assays are summarized below.



Nanowire FET coated with 'capture' molecules.



Conductivity through Nanowire FET is directly proportional to the Concentration of 'target' molecules at that instant.

**Time to results.** Immediate and Real-Time. Depending upon the experimental set-up the results are displayed immediately. For example, for a single sample applied by pipette, the signal will increase as a function of the on-rate until equilibrium has been reached. For typical antibody-antigen binding kinetics, equilibrium is reached in a few seconds at most. For continuous flow experiments, results are presented once per second and reflect the target molecule concentration at each second.

**Sensitivity.** Depending upon the charge density and size of the target molecule, it is not unusual to detect many biomarker molecules at the mid femtomolar to low picomolar range.

**Linearity/Dynamic Range.** Most target molecules are detectable in a linear range of four-five orders of magnitude. This permits measurement of different target molecules with widely divergent concentrations to be detected in the same sample. ELISA assays are generally linear for no more than two orders of magnitude.

**True Multiplexity.** Vista's NanoBioSensor System allows for the detection of proteins and DNA or RNA in the same sample. In addition, since there is no labelling required, the samples and target molecules therein can be re-used for other types of analysis such as electrophoresis or mass spec.

**DNA, RNA and Polymorphism Analysis.** Using PNA (Protein Nucleic Acid) probes, it is possible to quantify the concentration of target sequences of interest. In addition, Vista's Advanced NanoBioSensor allows for finely-tuned

temperature monitoring and control. By determining the melting temperature of bound duplexes, one can detect polymorphisms at the SNP level.

**Extensive Kinetic and Thermodynamic Information.** Since one can easily determine On- and Off-rates in Vista's NanoBioSensor using Vista's software, it is easy to determine the Equilibrium Dissociation Constant ( $K_D$ ) for any binding pair under a variety of temperatures and conditions. Since one can conduct binding experiments at two or more temperatures, the  $K_D$  can be further validated. In addition Gibb's free energy, enthalpy and other thermodynamic and kinetic parameters can be quantified.

**Wide Variety of Capture Molecules Usable.** Most antibodies work well as capture molecules as do Nanobodies, aptamers\*, single domain antibodies, SOMAmers, artificial antibodies and even receptors, ion channels and enzymes have been used successfully with Vista's NanoBioSensor System.

**Use of Tethered 'Target' Molecules to Identify Good Antibody and Vaccine Candidates.** 'Capture' and 'Target' are interchangeable when it comes to Vista's NanoBioSensing. For instance, by coating FET NWs with the Zika virus NS1 protein it is possible to detect binding of IgM antibodies, vaccine candidates and phage display candidates. With appropriate microplumbing and branch points whose gates are controlled by conductance signals, it would be possible to continuously flow a dilute phage display library over the nanowires and collect good candidates. With repetition under increasing stringency it would be possible to automate the isolation of the most promising antibodies, display phages, etc.

**Continuous Tissue Culture Monitoring.** Vista has developed a chip that can be immersed into cell or tissue cultures and continuously monitor the concentration of stress-induced proteins on a real-time basis for up to at least 48 hours.

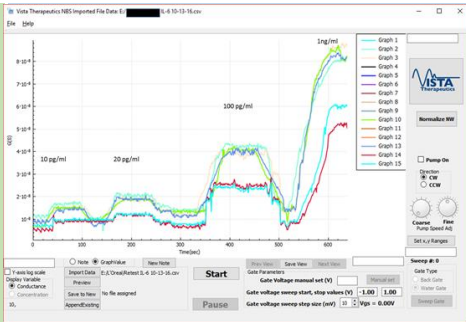

\*Because aptamers and SOMAmers have highly charged phosphate backbones, they are sub-optimal for quantitative studies.

**Build Your Own Assay.** Vista provides a capture molecule attachment kits that allow for the attachment of molecules with either aliphatic primary amine or aldehyde groups. It is inevitable that some capture molecules will work better than others for a particular purpose and it is generally worthwhile to test several candidate capture molecules.

**Re-Usability and Cost.** It is difficult to compare NanoBioSensing to any other assay. With most typical IgG-type antibodies with equilibrium dissociation constants of  $10^{-7}$  M or lower, target molecules can be washed off within a few seconds or minutes, depending upon conditions such as ionic strength, pH and temperature. Under these conditions, dozens of samples can be tested with a single NanoCard™. Depending upon the stability of the antibody and the storage conditions, a functionalized NanoCard can often be used repeatedly over a period of days and sometimes weeks. For smaller capture molecules such as Nanobodies where the most thermodynamically stable shape is assumed upon rehydration, a functionalized NanoCard can be dried and stored under nitrogen or argon for at least a year. Dried appropriately, they can be re-used many times. NanoBioSensing is the only method that permits real-time, continuous, label-free biomarker monitoring and there is no rational way to compare this ability against ELISA, lateral flow or RT-PCR assays. From the perspective of single sample comparison, the cost of sample preparation, the need for advanced lab capabilities, the time to results, the sample quantity required and the limited range of detection of these other methods, NanoBioSensing is as economically advantageous as it is scientifically superior.

**Summary.** Vista's NanoBioSensor System offers products and services for biomarker measurement than cannot be compared to any other assay system available anywhere else today. These products are available now and can be put use within days. Vista offers detailed product use instructions with each of its products and systems. If you have developed nanowire FET or Red-Ox applications of your own, Vista would be pleased to discuss how we can quickly adapt our systems with yours. Contact us at: [www.Vistananobiosciences.com](http://www.Vistananobiosciences.com) or [Info@Vistananobiosciences.com](mailto:Info@Vistananobiosciences.com). Call us at 505-474-3143.



Vista NanoBioSensor Features	Standard NBS Model	Advanced NBS Model (s)	Advanced 'Dip-Chip' NBS Model	Options
<p><b>Functions</b></p>	<p>All of Vista's NanoBioSensors (NBS) are designed and built as simple-to-use, integrated instruments that perform all of the functions required for using Field Effect Transistors (FETs) as biosensors as described in Charles Lieber's seminal paper (Nat. Protocols. 2006. 1:1711), but at a fraction of the complexity and cost of purchasing and integrating multiple, expensive components, writing software and firmware to collect and analyze the data, and then storing the data in such a way that it can be analyzed at a later date. The Standard NBS unit can also be used with Multi-Card NanoCards for measuring/monitoring more than one target molecule in the same sample as well as used with single Dip-Chips for immersion into cell cultures, bioreactors and some enzyme assays.</p>	<p>Advanced NBS unit performs all functions of Standard NBS unit but accommodates most NanoCard types including 'Thermo-Set NanoCards' with and without EEPROM data reading and recording capabilities. Thermo-Set NanoCards allow NW temperature to be monitored and controlled with both active heating and cooling capabilities facilitating thermodynamic analysis as well as melting temperature analysis for DNA polymorphisms micRNA quantitation and retrovirus species differentiation. May supplant RT-PCR for many uses. Heating facilitates complete dissociation of targets with slow off-rates, thus allowing rapid re-use in spite of tight binding. Integrated, reversible micro-pump controlled by software allows programmable switching between multiple sources. The Advanced NBS unit is a DSP version that allows for even greater sensitivity greater signal:noise ratios at low signal strength. Useful for single sample or flow through sample monitoring. Also compatible with single Dip-Chips and Multi-Chip NanoCards. Stainless steel housing permits decontamination with dilute H<sub>2</sub>O<sub>2</sub>.</p>	<p>Dip-Chip version of Advanced NBS model that can monitor up to seven separate 'Dip-Chips' in seven wells/biocultures simultaneously. Alternatively, seven different biomarkers can be measured in the same culture or bio-reactor. Frequency of analysis determined by end-user, depending upon the experimental design. Integration of multiple circuits into each Dip-Chip circuit allows for even greater sensitivity and greater signal:noise ratios at low signal strength. Permits use of cables to wells without 'antenna' effect noise. Custom lids and gaskets for 6-well plates that hold Dip-Chips in place minimize contamination.</p>	<p>Adaptor that allows NanoCard reader to use single Dip-Chip or multiple Dip-Chips. Adaptor and Faraday cable that allows Standard Dip-Chip to be used at up to two feet from the NBS unit. Adaptor assembly that allows single Dip-Chip to be used on a flow-through basis. NanoCard flow-through assembly for NW functionalization and flow-through applications.</p>
<p><b>Number of Circuits</b></p>	<p>Sample screening or continuous monitoring of up to 15 FET-gated channels simultaneously at 1 Hz (1 measurement per second) for up to 48 hours.</p>	<p>Sample screening or continuous monitoring of up to 15 FET-gated channels simultaneously at 1 Hz (1 measurement per second) for as long as the NW FET sensors are performing well (frequently up to 60 hours).</p>	<p>Sample screening and monitoring of up to 15 FET-gated channels simultaneously at 1 Hz (1 measurement per second). Up to 7 tissue culture wells or bio-reactors can be monitored simultaneously. Multiple 'Dip-Chip' NBS units can be integrated for additional circuits.</p>	<p>Converter that allows field use of NBS units with 12 V power sources such as cigarette lighter outlets or rechargeable battery packs.</p>
<p><b>Software and System Functions</b></p>	<p>Vista software collects, interprets and displays data on a real-time basis on a log or linear basis. It allows the user to display any or all circuits and updates conductance ( or concentration) values once each second. One can reload old files and quickly find the exact value of any circuit at any time for flow-through runs as long as 48 hours. It can run a sweep through a watergate at any time to confirm good FET function at any time or to allow for comparison with previous runs or different circuits/normalization between different circuits. It is compatible with 15 circuit NanoCards or two circuit Dip-Chips. It will build standard curves with known concentrations and then use that curve data to show biomarker concentrations on a real-time basis and much more.</p>	<p>In addition to the functions provided by the standard software, the advanced software, in conjunction with NanoCard temperature control, will provide many sophisticated analyses of thermodynamics, kinetics, binding rate constants, equilibrium dissociation constants, reports temperature at 1 Hz, controls both heating and active (Peltier) cooling, determines flow rate past nanowire array based upon pump speed and tubing ID. It calculates time required for sample to reach NWs based upon the above inputs. Reversible pump allows for mixing in the NW chamber and overcome laminar flow-based errors. It allows users to choose circuits to be monitored or have the computer eliminate circuits based upon user-defined criteria. It is compatible with all NanoCards. With adaptor is also compatible with Dip-Chips. Allows frequency of datapoint collection to be determined by user. It is compatible with all NanoCard and Dip-Chip types. Allows frequency of data collection to be determined by user. Develops standard curves, curve-fitting, statistical analysis, normalization, and outputs data in conductance or concentration. Voltage sweeps through watergate at any time. Controls optional source switching system. Performs multiple graphing options.</p>	<p>The 'Dip-Chip' NBS software also allows one to color code the circuits from each Dip-Chip circuit differently so that at a glance one can follow the signal from any particular well or sample.</p>	
<p><b>Price</b></p>	<p>\$29,500 plus shipping and handling. Usually ships within three weeks of receipt of payment.</p>	<p>Advanced NBS unit \$55,900 plus shipping and handling. Available early 2017.</p>	<p>\$55,900 plus shipping and handling. Available early 2017.</p>	<p>See NanoCard and Functionalization Sheet for more details and pricing.</p>
<p><b>Images</b></p>				
<p>NOTE: Units sold for research purposes only. Not to be used for commercial services without separate agreement with Vista. No component can be altered, repaired or removed by buyer; any such attempted alteration will void warranty. Key components 'potted' to prevent attempted reverse engineering.</p>				

NanoChips, Dip-Chips and Functionalization Kits.	Standard NanoCard	Heated, Cooled and Temperature Monitored 'Thermo-Set' NanoCards	Heated, Cooled, Temperature Monitored and EEPROM Card-stored data NanoCards 'Thermo-Set Memory Cards'	Dip-Chip NanoCards'	Options
Basic Functions	<p>Vista's Standard NanoCard consists of up to 15 circuits. Although fabricated in a top-down manner, Vista's proprietary chip design and fabrication process typically results in no fewer than 10 closed circuits per chip. Each chip has a watergate that is equidistant to all source and drain electrodes and therefore generates the same field strength relative to each nanowire allowing it to serve as a quality check of the FET-gated circuits at any time from -2.5 to +2.5 V at 10 to 100 mV/sec. There is also a back gate for sweeps at higher voltages. Each NanoCard comes with a print-out of a watergate sweep to show which are the good circuits and what their performance is. NanoCards can be made with P-type, N-type or both P- and N-type nanowires.</p>	<p>Conductance and transconductance through FET gated NWs are strongly influenced by temperature. In order to obtain consistent data, to conduct thermodynamic experiments, quantify micRNA or to detect DNA SNPs it is necessary to control the temperature of the sample at the NW surface, Vista's 'Thermo-Set' NanoCards actively heat and cool the chip surface. The temperature is reported for each measurement and is controlled by Vista's software. Temperature ranges are sufficient for measuring SNP, micRNAs as well as for conducting a variety of kinetic and thermodynamic experiments.</p>	<p>Thermo-Set Memory Cards Perform all functions of Thermo-Set NanoCards, but inclusion of an EEPROM data storage chip captures and stores certain user-selected data right on the card itself, such as water gate sweep data before and after an experiment, or experimental design employed to generate the data.</p>	<p>As their name implies, 'Dip-Chip' NanoCards are designed to be immersed into a fluid sample of interest, such as tissue culture wells, bioreactors, enzyme reactions or water sources. Dip-Chips can be used for up to 72 hours to continuously monitor the presence or concentration of a biomarker or virus. They can also be used as simple one-time quick measurement devices for field detection of biomarkers or viral pathogens in human or animal bodily fluids. Vista's Advanced NBS unit can monitor up to six separate 'Dip-Chips' in six different locations simultaneously, such as cell culture wells or biocultures or small-scale bio-reactors.</p>	<p>Adapter that allows NanoCard reader to use single Dip-Chip. Adapter and Faraday cable that allows Dip-Chip to be used at a distance from the NBS unit. Adaptor assembly that allows single Dip-Chip to be used on a flow-through basis. NanoCard flow-through assembly for NW functionalization and flow-through applications.</p>
Price	<p>Standard NanoCards. Five for \$1200.00 plus Shipping and Handling. Usually ships within five working days. Comes with one flow-through set-up and five gaskets. Additional Reusable Flow-Through set-ups, five for \$750.</p>	<p>'Thermo-Set' actively cooled and heated NanoCards with on-card EEPROM memory. Depending upon stability of capture molecule and storage conditions, many NanoCards can be used repeatedly. Five for \$2,000. Currently back-ordered. Available early 2017.</p>	<p>Functionalization Kits for P and N type NWs. Available for attachment through aldehyde or lysine groups (good for ten Dip-Chips or NanoCards), \$500 plus shipping and handling.</p>	<p>'Dip-Chips'. Ten for \$3,000. plus S &amp; H Allow two weeks.</p>	<p>'Dip-Chips'. Ten for \$3,000. plus S &amp; H Allow two weeks.</p>
Images					

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