LAMDAGEN
CORPORATION

LightPath™ 54

Enhance your research with real-time monitoring and quantitation of biomolecular binding with highly sensitive detection in a label-free format

Enhance Your Protein Interaction Research with A New Level of Bench-Top Productivity

LamdaGen's **LightPath™ S4** system performs highly sensitive, label-free, real-time monitoring and analysis of biomolecular binding events. The S4 system integrates advanced optics, proprietary software and powerful nano-structured biosensors in a simple to use, flexible, and economical hands-on platform ideal for **rapid assay development, kinetic profiling, sensitive protein quantitation** and **characterization of protein-ligand interaction.**

The LightPath™ S4 puts power on the bench-top delivering content-rich data that streamlines workflows and generates fast and precise results – even in crude media. The system requires micro-volume samples to generate kinetic profiles, quantitative information, enzyme activity measurements and much more, allowing researchers to make earlier informed decisions.

Performance

High quality data and rapid results

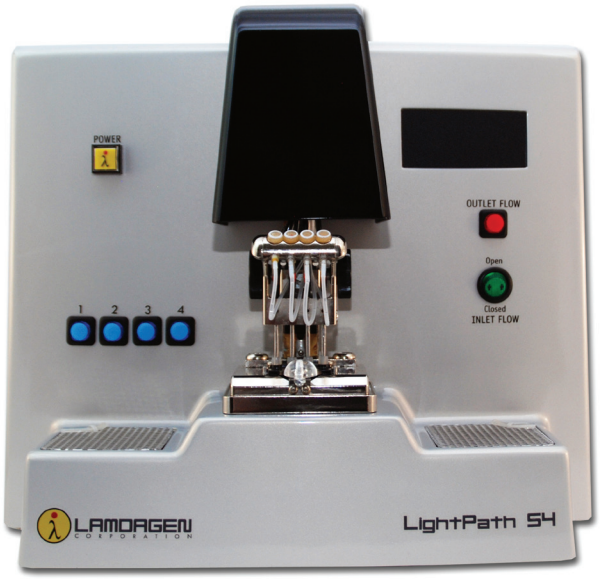
- Label-free, real-time
- Highly reproducible and robust biosensors
- Kinetic and affinity data
- Directly analyze targets in complex media
- Powerful software for data acquisition and analysis

Economical

Efficient and affordable system

- Assay development in hours vs. days
- Eliminate purification steps
- Broad dynamic range reduces dilution steps
- Easily regenerate sensor surfaces
- Small sample volumes
- No costly service contracts

Economical
Sensitive
Fast



LightPath™ S4

Versatile

Hands-on plug and play

- Easy to learn and operate
- No dedicated operator required
- Pre-functionalized sensors
- Quickly alter experimental conditions
- Perform analysis easily at your bench-top

Antibody Characterization

The LightPath S4 is a versatile system for rapid antibody characterization. Specific binding events on the surface of the P4 biosensor are precisely monitored in real-time with LamdaGen's RT-PEAK Acquisition Software. Antigens are supplied in buffer, serum, lysate or other crude media and the wavelength shift resulting from the binding event is displayed as a response curve vs. time (sensorgram). Kinetic and affinity data can be readily obtained without extensive data post-processing.

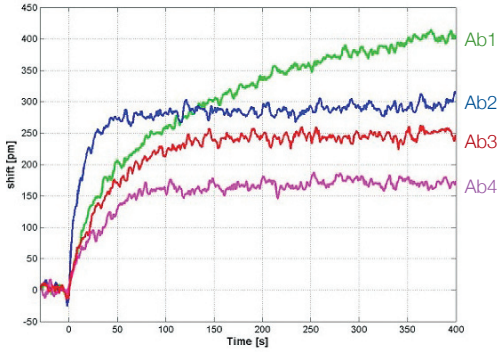
- Quickly rank candidates according to their binding affinities, specificity and cross reactivity
- Accurately perform epitope binning, antibody pairing and kinetic studies
- Directly analyze in crude media – no purification steps, labeling, or secondary reagents needed
- Run 4 independent parallel assays
- Perform quick inhibition/competition assays

Antibody Pairing

Surface / Analyte	RPab 1	RPab 2	MsMab 2
RPab 1	320 25	225 65	290 → w/antigen 31 → w/o antigen
RPab 2	400 22	nd 100	**420** **30**
MsMab 2	nd 28	nd 40	145 5

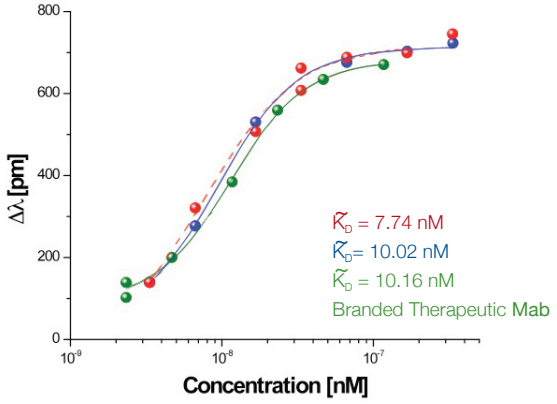
Identification of optimal antibody pair - MsMab 2/RPab 2 - highest signal, low background. Delta lambda in process media after 15 minutes of reaction. nd: not detected

Antibody Ranking



Antibody kinetic ranking: By slope: Ab2 > Ab1 > Ab3 > Ab4
By shift: Ab1 > Ab2 > Ab3 > Ab4
Fast antibody affinity screening on ligand-immobilized biosensor – Time elapsed: 7 minutes. Raw data shown.

Kinetic Studies



Kinetic calculations (K_D): comparison between a branded antibody (green) and two biosimilars (red and blue)

LightPath S4 Applications

Assay Development
- Immunoassay
- Chemical assay
- Enzymatic assay

Antibody Characterization
- Antibody/ligand affinity screening
- Epitope binning
- Hybridoma selection and ranking
- Anti-idiotypic anitbody screening
- Antibody titer
- Fragment screening

Kinetic Studies

Process Development
- Antibody selection
- Process optimization assays
- Protein expression monitoring

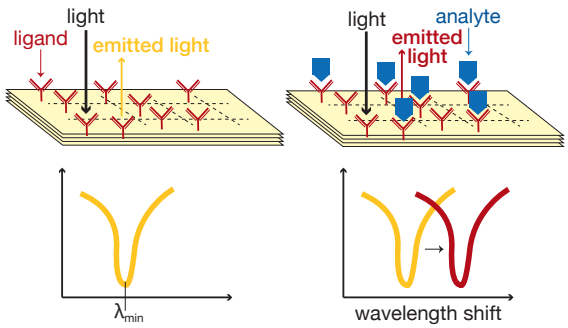
Manufacturing & QC
- Process monitoring
- Contaminant detection
- In-line and off-line QC

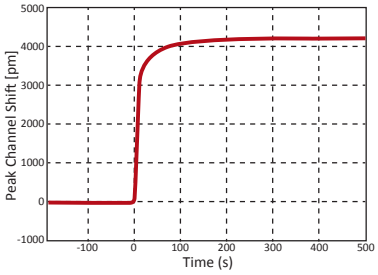
Pre-Clinical and Clinical Diagnostics
- Kinetic profiling
- Biomarker assays
- Point of Care (POC)

Localized Surface Plasmon Resonance

LamdaGen's Localized Surface Plasmon Resonance (LSPR) platform is based upon its nanostructured metallic thin-film surfaces. These patented surfaces form a powerful biosensor to detect real-time biomolecular interactions through precise monitoring of wavelength change at its surface.

LamdaGen's LSPR requires a light source and its nano-structured LSPR biochip. There is no need for adaptive elements to couple the light to the localized plasmons of the nanostructured surface. Among the broad spectrum of light, one wavelength resonantly couples to the localized plasmons on the biochip surface and is dominantly absorbed. Therefore, the monitored reflected light has a minimum at this specific wavelength, called λ_{min}. The value of λ_{min} is dependent on the dielectric constant at the sensor surface. Changes at the surface as those induced by the binding of biomolecules, cause a change in λ_{min} which is precisely monitored by the LightPath S4.



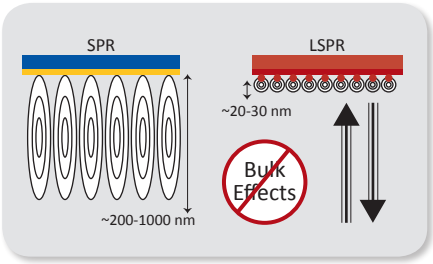


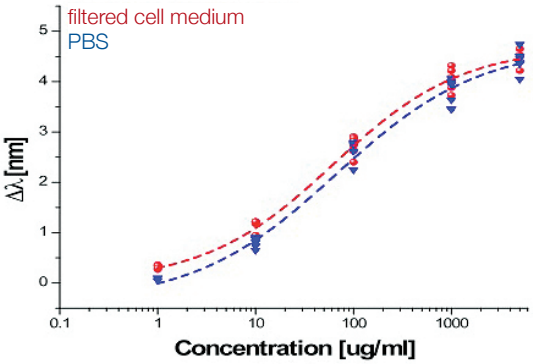
Response curve vs. time (sensorgram)

The plasmon is sensitive to changes in the index of refraction as a binding event occurs on the LSPR surface.

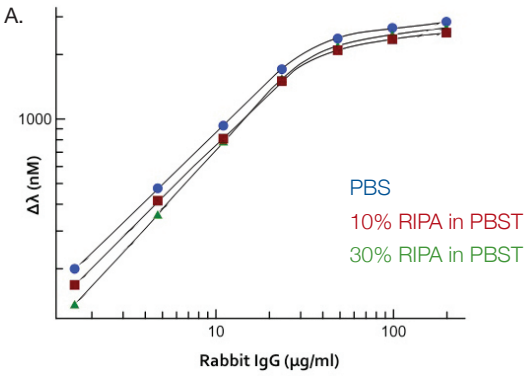
Matrix Compatibility

One of the fundamental differences between LSPR and SPR is the distance from the surface at which the respective plasmons can detect changes. While the penetration depth of the plasmon field for SPR is between 200-1000 nm, it is approximately 15-30 nm for LSPR. Hence, LSPR is far less susceptible to bulk effects occurring away from the surface. In other terms, LamdaGen's LSPR detects changes very near the LSPR surface thereby allowing compatibility with complex or crude media.





The binding properties of rabbit IgG to protein A were measured in PBS and in filtered cell medium.



B. Percent signal reduction in RIPA buffer

Rabbit IgG (µg/ml)	1.6	4.7	11	23.5	48.5	98.5	198.5
10% RIPA in PBST (%)	15.85	12.5	12.95	11.93	11.81	11.45	10.98
30% RIPA in PBST (%)	31.69	24.55	15.42	9.29	7.61	6.74	6.52

Binding of rabbit IgG to protein A/G in RIPA_PBST and PBST control (A). Percent signal reduction in RIPA_PBST compared to PBST (100%) (B).

Accelerate Assay Development and Optimization

The LightPath S4 is uniquely suited to accelerate assay development and move rapidly from concept to optimized assay. Fast and flexible multi-parameter testing happens in real time and guides efficient selection and optimization of immobilization chemistry and binding conditions while conserving valuable sample.

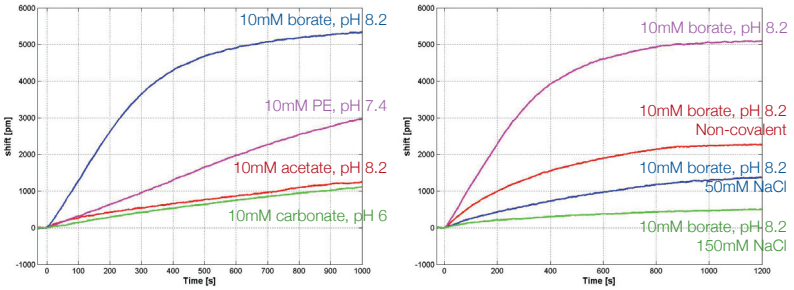
LightPath S4's speed and flexibility combined with its robustness and ease of use make it ideal for accelerating step-by-step assay development.

- Develop assays in hours vs. days
- Quickly investigate multiple assay conditions (e.g. pH, ionic strength) to identify optimal binding conditions
- Optimize ligand immobilization level
- Rapidly identify antibody pairs and cross reactive reagents
- Gain richer, real-time binding data vs. traditional end point assays
- Use pre-functionalized biosensors for rapid set up

* Raw data shown

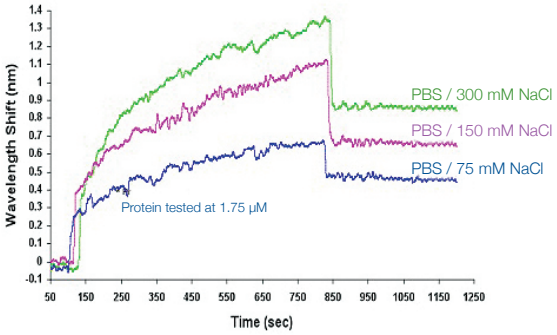
Optimize Conditions:

Ligand Immobilization Chemistry*



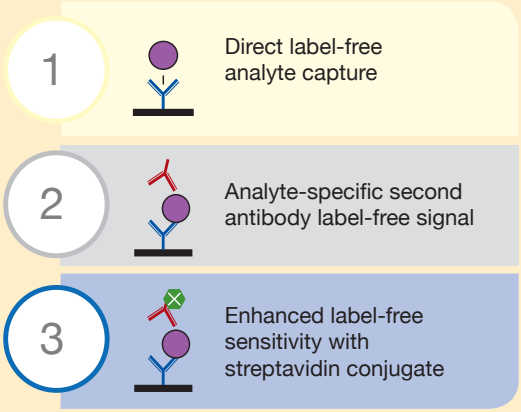
Optimization of ligand immobilization conditions: buffer pH (left) and ionic strength (right).

Protein Binding*



Optimization of protein binding conditions. His-tag protein shows greater binding activity to Ni-NTA biosensor at higher salt concentrations.

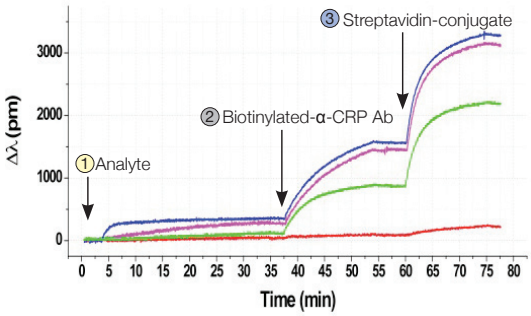
Label-free Signal Enhancement



Sequential signal enhancement in label-free format.

3-Step Immunoassays – *Label-free**

Ligand Ms α-CRP Ab	+	+	+	MsIgG
Analyte CRP (nM)	0.4	2	20	20
Biotin-α-CRP Ab (µg/ml)	100	100	100	100
Streptavidin-conjugate (µg/ml)	10	10	10	10



CRP binding to anti-CRP Ab immobilized biosensors and subsequent signal enhancement with biotinylated-α-CRP Ab and streptavidin-conjugate.

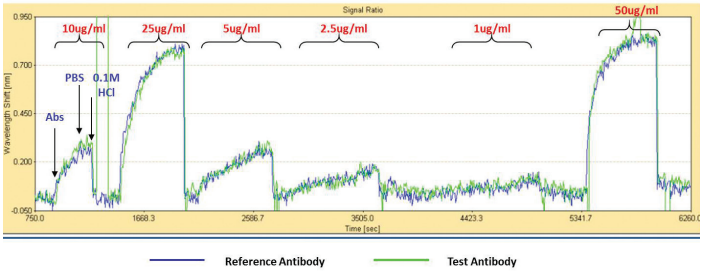
Powerful Platform – Many Applications

The LightPath S4's versatility extends to many application areas where rapid, real-time results are essential. The system quickly identifies specific molecular interactions of non-labeled molecules such as antibodies, proteins or low molecular weight compounds. By rapidly exposing the nature of complex biomolecular interactions, researchers gain valuable insights into the nature of biological systems and disease processes.

In addition to antibody characterization and quantitation, affinity studies and rapid assay development, the LightPath S4 easily performs:

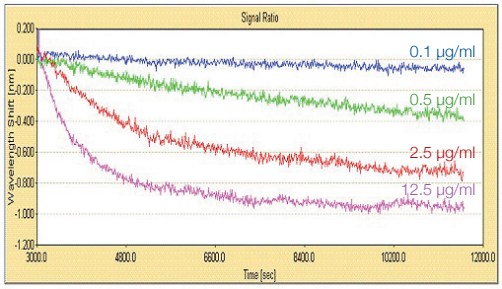
- Detection and quantitation of
  - Bacteria
  - Viruses
  - Toxins
  - Small molecules
- Monitoring of
  - Enzymatic activities and inhibition
  - Chemical reactions

Biosimilar Affinity Evaluation*

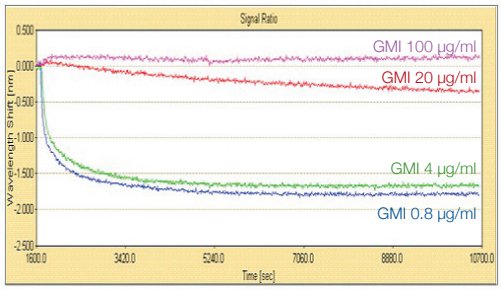


Biosimilar candidate antibody benchmarked against reference antibody at different concentrations. Valuable results from studies can be translated to QC protocols for the manufacturing suite.

Protease Activity & Inhibition*

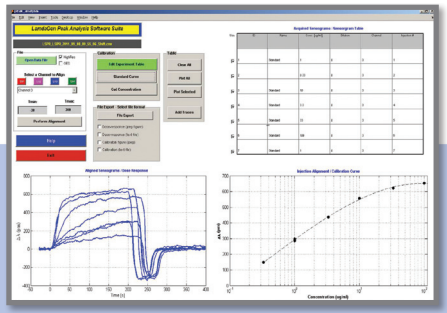


Monitor trypsin activity in real time on casein immobilized biosensor.



Inhibition of trypsin activity (100 µg/ml) on casein immobilized biosensor by Glycine Max Inhibitor (GMI) in a dose dependent manner.

** Raw data shown*



PEAK Software

Powerful software for fast, easy data acquisition and analysis

The LightPath™ S4 is equipped with LamdaGen's RT-PEAK Acquisition and PEAK Analysis Software for real-time data acquisition and analysis, quality graphing capabilities and logical analysis templates. Data is available for customized analysis and model integration. The software enables fast data processing and integrates various fit models to generate association and dissociation constants, calibration curves to allow quantitation of unknowns, and much more.

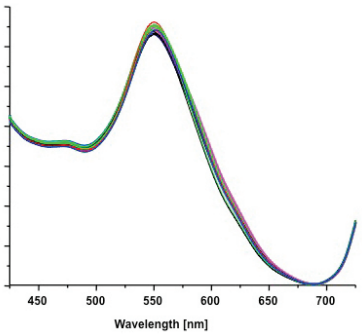
Reproducibility You Can Depend On

LamdaGen couples advanced physics, chemistry and biology to bring nanotechnology to biotechnology

LamdaGen's nano-structured P4 LSPR biosensor is the heart of the LightPath S4 system. LamdaGen has developed a proprietary nano-manufacturing process to reproducibly manufacture robust metallic biosensors that allow precise real-time monitoring of molecular binding events. The rich data derived from the highly sensitive P4 biochips enable scientists to make better and earlier decisions in their research.

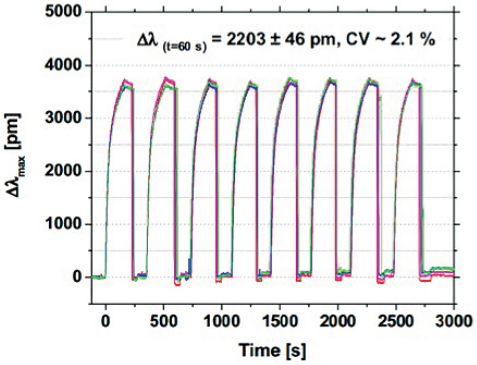
LamdaGen offers a range of pre-functionalized sensors for different assay models. Commonly, the sensor surface is covered with a self-assembled monolayer (SAM) formed by a mixture of hydrophobic and hydrophilic n-alkanethiols. The hydrophilic alkanethiols are terminated with a variety of functional groups, as listed in the table.

Biosensor Reproducibility



Highly reproducible surfaces are illustrated by overlaying plasmons from 20 sensor chips, 4 wells per chip, total of 80 plasmons.

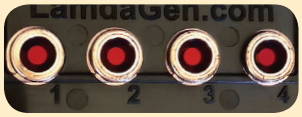
Bioassay Reproducibility



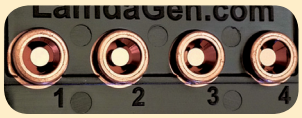
Reproducibility of IgG binding and regeneration on a protein A biosensor (using 2mM HCL).

P4 Biosensor

Strong interaction between light and the nanostructured surface is responsible for the intense burgundy color of LamdaGen's pure gold LSPR surfaces.



LamdaGen Nano-Structured Gold



Planar Gold

LAMDAGEN P4 SENSOR SURFACES

Sensor Chemistry	Description
Blank	Non-functionalized surface
Carboxyl	-COOH groups are activated with EDC/NHS to form covalent binding with -NH2 on the ligand
Amine	-NH2 group allows forming covalent binding with -COOH on the ligand via amide bond activated by EDC/NHS
Epoxy	Reacted with the -NH2 on the ligand without the need of a cross-linker (for working conditions at pH9)
Aldehyde	Reacted with the -NH2 on the ligand without the need of a cross-linker (for working conditions at pH9)
Thiol	-SH on the surface to link to -NH2 on the ligand through SMCC-like bioconjugation approach
Glutathione (GSH)	Ideal for capture of GST-tagged proteins
Protein A	Binding with high affinity to Fc region of human IgGs. Other IgG species may vary.
Streptavidin	High binding affinity with biotinylated molecules, including peptides, oligonucleotides, proteins and sugars.
NTA	Ideal for capture of polyhistidine-tagged recombinant proteins. Histidine tagged proteins will bind to the NTA in a defined orientation.

Custom surfaces are available.
Contact us at info@lamdagen.com

LightPath™ S4 System Specifications

Detection Technology	Optical sensing based on Localized Surface Plasmon Resonance (LSPR)
Biosensor Type	Disposable, single-use, nano-structured metallic chips
Information Provided	Kinetic and affinity analysis (k_{obs}, K_a, K_d, K_D), Concentration monitoring, Quantitation
Sample Types	• Proteins • Antibodies • Peptides • Media containing serum • Buffers containing DMSO • Periplasmic fractions • Untreated cell culture supernatants • Crude cell lysates
Sample Volume	15 – 60 µl per assay
Quantitation Range	For Human IgG: <0.1 µg/ml – 5.0 mg/ml

Throughput	4 independent parallel channels
Analysis Temperature	Ambient
Drift	5 pm/hr
Noise	< 1 pm
Peak Analysis Software	• Rapid screening • Specificity • Cross-reactivity • On/off rates • Epitope binning • Concentration monitoring • Binding-site competition • Kinetics
Data Display	Peak real-time trace software
Physical Specifications	13” H x 14” W x 13” D (33cm H x 36cm W x 33cm D)
Weight	20.5 lbs (0.3 Kg)

For quotes or additional information about LamdaGen’s LightPath S4 platform for label-free, real-time detection of biomolecular interactions:

Contact us at info@lamdagen.com
Visit us at www.lamdagen.com

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