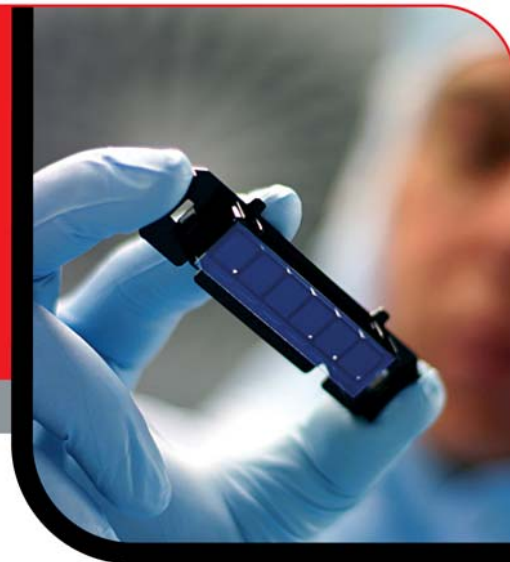


FAQ

for ZeptoMARK Reverse Phase Protein Arrays



Can I purchase everything I need to perform reverse array analysis from Zeptosens?

Yes, Zeptosens provides not only a complete reverse array analysis platform but also all the reagents, chips and protocols required. As a full solution provider, we support our customers for both technical and biological applications.

Chip-Related Questions:

How many samples fit on your arrays?

We can fit 32 cell lysates on each array with each cell lysate spotted in 4 concentrations and in duplicate. Alternatively, we can spot 64 cell lysates with each cell lysate spotted once in 4 concentrations. One ZeptoMARK chip contains six arrays.

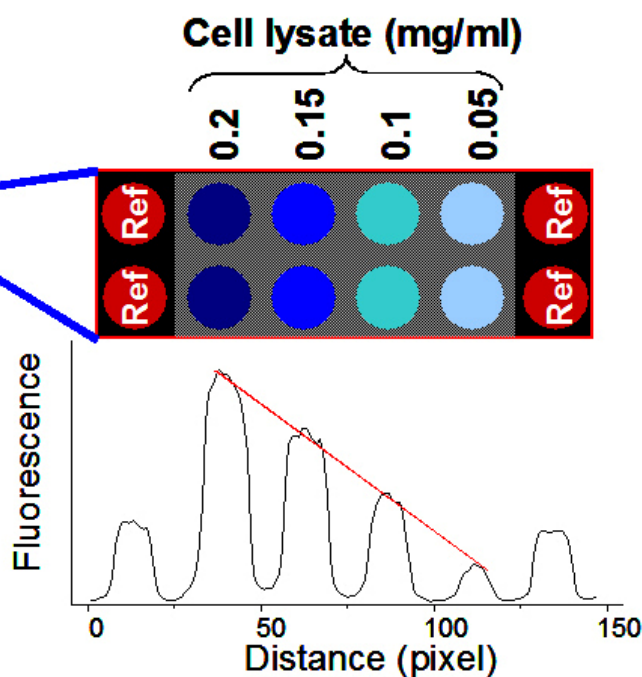
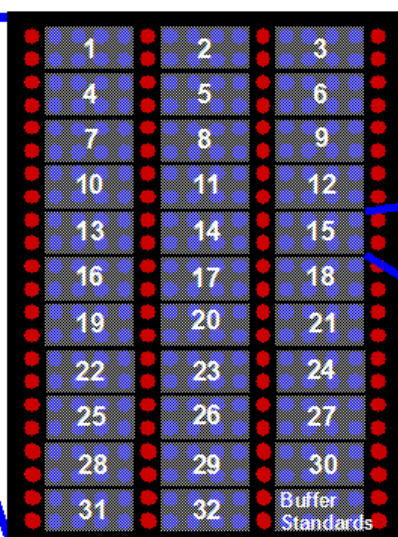
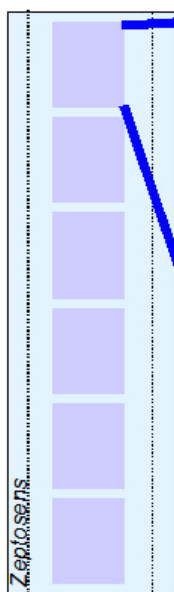
How many data points (=spots) are on your chips?

Each ZeptoMARK chip is arranged in six arrays, each with 256 positions for sample deposition, so in total 1536 sample data points can be collected per ZeptoMARK chip (not including the data points used for referencing and quality control).

6 Arrays / Chip

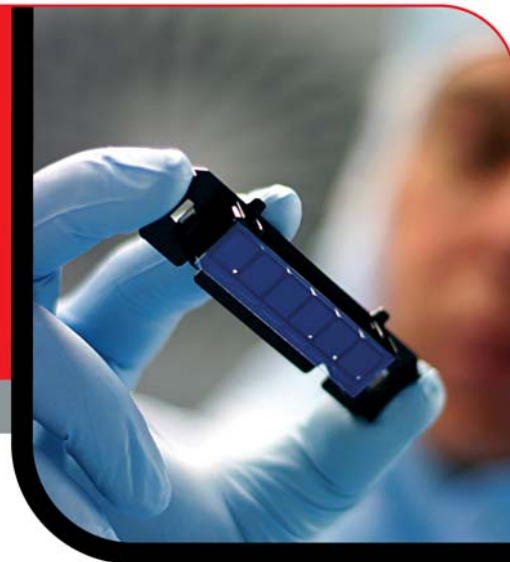
32 Cell Lysates / Array

Duplicates and 4 Dilutions / Cell Lysate



FAQ

for ZeptoMARK Reverse Phase Protein Arrays



How do you read the arrays/ZeptoMARK chips?

ZeptoMARK chips are placed in the ZeptoCARRIER, which holds a maximum of six ZeptoMARK chips. It is the ZeptoCARRIER which is inserted into the ZeptoREADER. The stacker function inside the ZeptoREADER allows loading a maximum of ten ZeptoCARRIERS in a single run, for a total (maximum) run of 92,160 data points in six hours.

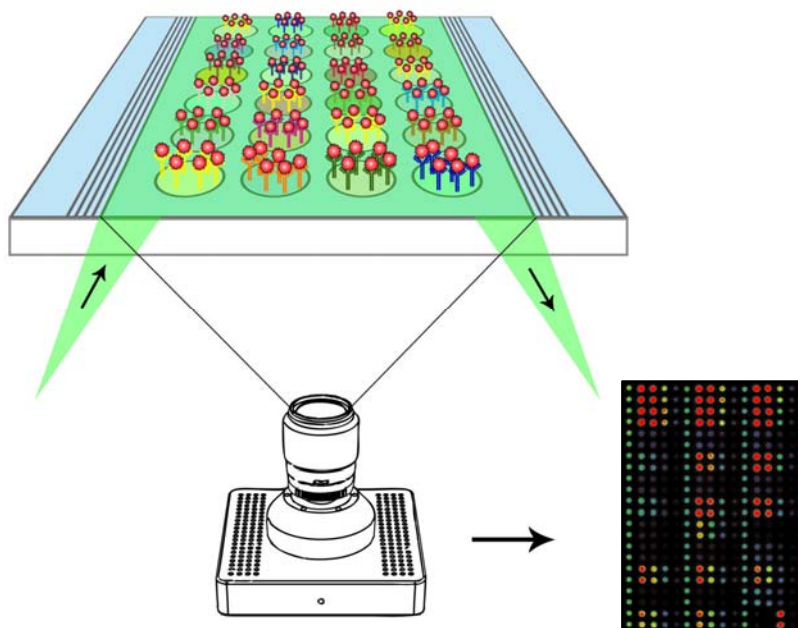
Why do you spot the sample lysates in serial dilutions?

By using four concentrations of every sample, we can determine an on-chip dose response curve. This acts as a quality control feature for the assay, since we can directly check if the assay is performed within its linear range.

ZeptoREADER-Related Questions:

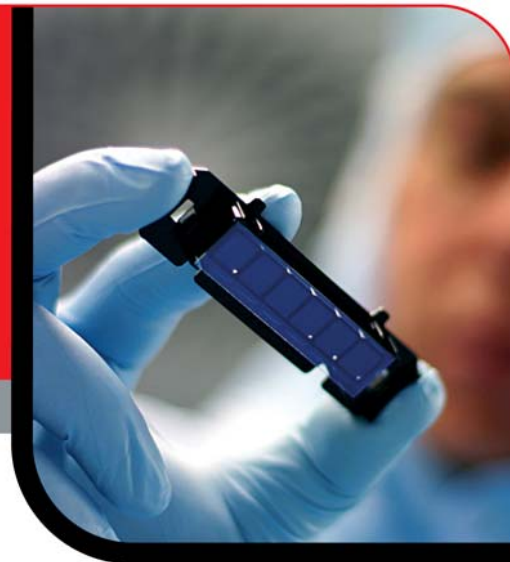
How does Planar Waveguide technology work?

This is a key component of how the Zeptosens platform achieves its unique sensitivity. Gratings on each side of the glass slide allow coupling of the excitation light into the wave-guiding layer across the surface of the ZeptoMARK chip, illuminating only the protein bound to the surface, and drastically reducing or eliminating background.



FAQ

for ZeptoMARK Reverse Phase Protein Arrays

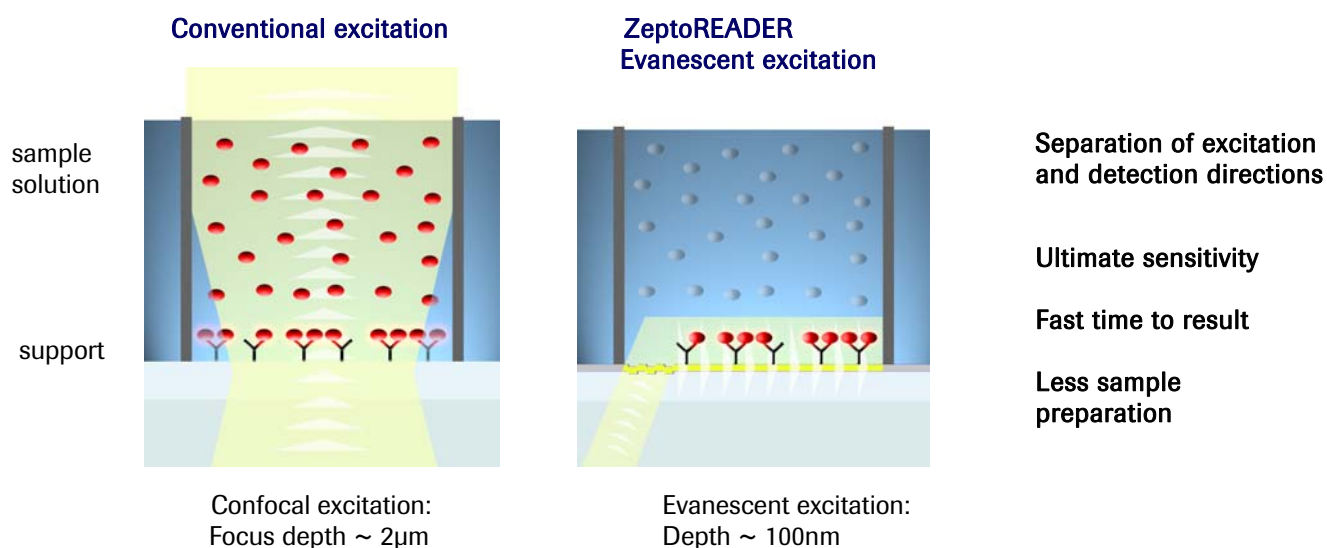


What types of fluorophores can be used with this system?

In the Zeptosens reverse array approach we typically use fluorophores with an excitation wavelength of 635nm. In addition, the ZeptoREADER also allows for detection of fluorophores excited at 532nm.

Why are planar waveguide based chips superior?

Using planar waveguide technology, light is propagated along the chip surface, illuminating a depth of about 100 nanometers. This results in detection of surface-confined molecules only. As a consequence, the signal-to-noise ratio is significantly improved compared to conventional optical detection methods.

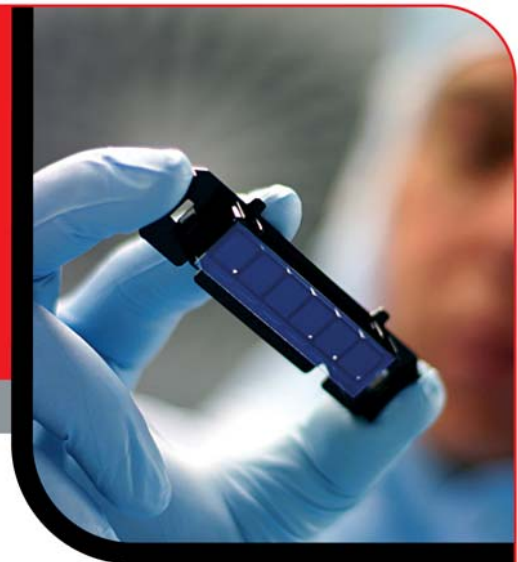


Do you make use of an enzymatic signal amplification to achieve your detection sensitivity?

No, the superior sensitivity of planar waveguide technology allows for straightforward fluorescence detection without the need for enzymatic signal amplification, even for detection of low-abundance molecules.

How are the sample lysates immobilized on the ZeptoMARK chip?

By using a highly hydrophobic surface coating on ZeptoMARK chips, we can ensure effective adhesion of the proteins in the sample lysates to the surface of the ZeptoMARK chip.



FAQ

for ZeptoMARK Reverse Phase Protein Arrays

Can any type of chip be used with the ZeptoREADER?

No, our extraordinary sensitivity is achieved in conjunction with our planar waveguide based ZeptoMARK chips. Ordinary glass slides or nitrocellulose slides cannot be used with this platform.

Spotting Questions:

How much sample material is in a single spot?

A single spot on an array is the result of the deposition of a 400 pL droplet from a lysate with a protein concentration between 0.05 and 0.2 mg/ml. This corresponds roughly to the protein content of a single cell.

What kind of spotting equipment does Zeptosens use and why?

Zeptosens has obtained excellent results with non-contact spotters in combination with our ZeptoMARK buffers. We prefer the reliable piezo-electric NanoPlotter® by GeSiM, which Zeptosens especially configures for ZeptoMARK reverse arrays.

Does Zeptosens also sell spotting equipment?

Zeptosens both uses and sells NanoPlotter® microarray spotters. Optimal workflow solutions are provided by integrating the NanoPlotter® into the ZeptoMARK protein array system.

What is the reproducibility of spotting lysates?

Replicate spots of lysate samples with the NanoPlotter® on ZeptoMARK chips typically achieve a CV < 3% within a spotting run.

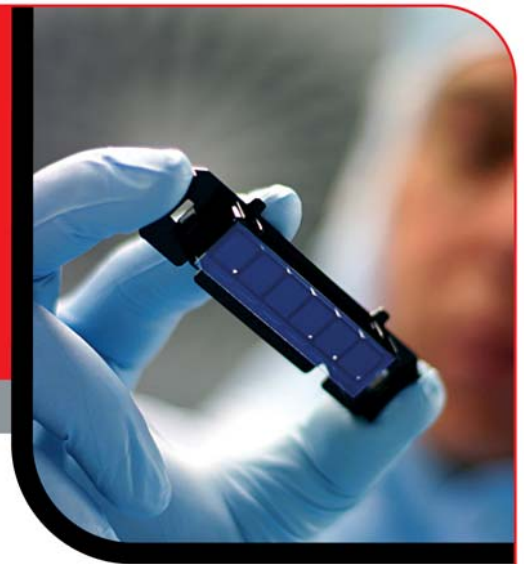
Study Services Questions:

Can I expect support from Zeptosens in the design of a reverse array study?

When a study is requested from Zeptosens, we offer our assistance with study design and antibody selection. Our extensive experience in the field of reverse phase protein arrays has proven to be extremely helpful to our customers, saving both time and money in research initiatives.

FAQ

for ZeptoMARK Reverse Phase Protein Arrays



How much time will it take to perform a study and to receive results?

Zeptosens routinely delivers small scale study results in ten to fifteen working days after receiving lysate samples which comply with our specifications. The size of the study can also impact the scheduling of your study, for example, hundreds of samples and hundreds of proteins will extend the completion time of your study.

What types of sample materials are acceptable for reverse arrays using the Zeptosens platform?

We have tested samples from a wide variety of biological origins. Although most studies are requested for cell lines, we can also perform studies with primary cells. Organs or tissue extracts are also popular where we routinely analyze lysates from (needle) biopsies, xenografts, transplants, and cryo-sections. For studies involving analysis of body fluids, some special requirements maybe needed and we suggest you contact us for further discussion for optimal design of your study.

Is it possible to analyze lysates from mouse or rat origin, or is the system restricted to material from human origin?

Zeptosens' reverse protein array system works well with samples of mouse or rat origin. While our antibodies are all validated for human proteins, the vast majority also works perfectly fine on mouse and rat derived samples.

How much sample material is needed to perform a study?

For a routine study we require for each sample a minimum of 50 microliters of a lysate at a protein concentration of 2-4 mg/ml in our lysis buffer CLB1. This can be easily obtained from biopsy material, xenografts or cultured cell lines.

Alternatively, for cells grown in 96 well plates, the CLB96 lysis buffer should be used. In that instance, we require at least 60 microliters of a lysate with a protein concentration of 0.3-0.4 mg/ml.

What is the minimum protein concentration required for your samples?

Zeptosens requires a minimum total protein concentration of 2 mg/ml for samples lysed in CLB1 buffer, determined by using a modified Bradford test according to the protocol provided by Zeptosens.

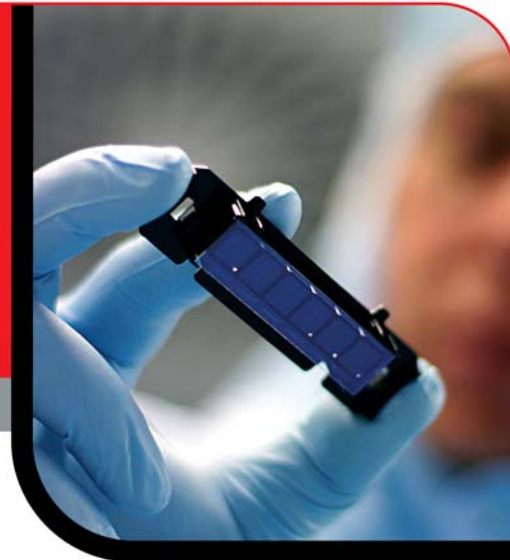
A minimum protein concentration of 0.3 mg/ml is required for samples lysed with CLB96 buffer. As with the CLB1 buffer, we will supply the buffer and all the required protocols.

Is there an optimal number of samples for an experiment?

Up to 32 samples at four concentrations in duplicate fit on one array. While we can always accept a lesser number of samples, the chip layout is arranged for 32 samples or multiples thereof. Typical studies involve sets of 32, or multiples of 32 samples.

FAQ

for ZeptoMARK Reverse Phase Protein Arrays



How many antibodies should I select?

Every sample on an array will be probed with a single antibody against a chosen analyte. Currently we have [225 validated antibodies](#) suitable for reverse protein arrays. With the Zeptosens [Reverse Array process](#), we can use an almost unlimited number of validated antibodies.

What do we receive if we request a protein profiling study?

Once a study has been completed, Zeptosens prepares a report containing the analysis of the results as well as the data in Excel format. We send this report to the customer and schedule a conference call to review the results and answer any questions the researcher may have after taking time to evaluate the study results. All study information and results are confidential and remain the property of the customer.

Assay-Related Questions:

What should I do if the antibody I am interested in doesn't appear on your list?

Check with us via email or a phone call – we are constantly updating our lists.

Specially developed antibodies or your own antibodies can be used provided they have been previously validated by single band Western blots. If this has not yet been done, Zeptosens can validate them for you. Once the validation is accomplished, the study can proceed.

How does reverse protein array technology compare in terms of reproducibility and reliability to classical ELISA-based methods?

A recent peer-reviewed publication offers comprehensive data on robustness and reliability (Pirnia *et al.* Proteomics 9, 3535-3548, 2009).

The CV for assay variation of most ELISA assays tends to be between 10-15%, and the variation between biological samples well above 20% (Liu, *et al.*, Clinical Chemistry 51,1102-9, 2005).

A direct comparison between ZeptoMARK reverse arrays and ELISA has been published. (van Oostrum *et al.* Proteomics Clinical Applications 3, 412-422, 2009).