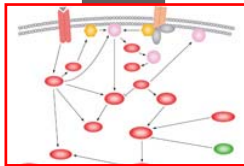
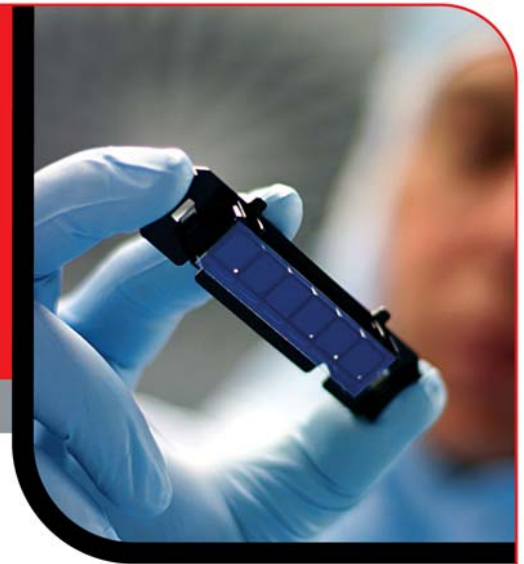


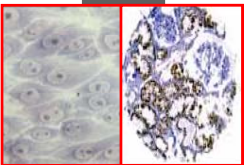
ZeptoMARK Reverse Arrays

Process for Multiplexed Pathway Activity Profiling Services



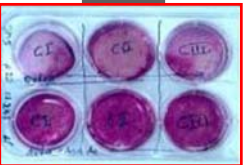
Experiment Design

Signaling pathways to be monitored and antibodies to be selected are jointly defined by Zeptosens and the customer to optimally design the study with respect to monitoring of protein expression and activation.



Cell Cultivation

Mammalian cells are cultivated in flasks or microplates by the customer. Current reverse array standard procedures require a minimum of 10^5 cells or an equivalent amount of tissue. In selected studies, good results can be obtained with as little as 25'000 cells when high-affinity antibodies are available.



Cell Treatment

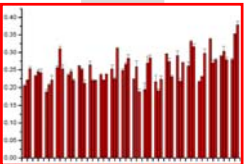
Cells are treated with inhibitors or other reagents in time or concentration series to generate data for biomarker validation, toxicology studies, signal propagation investigation or validation of systems biology models.



Lysis of Cells or Tissues

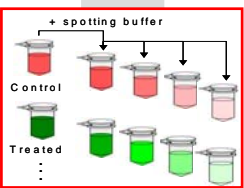
Treated and control cell samples as well as tissue material in an equivalent amount are lysed using ZeptoMARK reverse array lysis buffer and protocols. Due to denaturing and enzyme inhibiting properties of the lysis buffer, the biological processes within the cells are effectively frozen.

Freeze and Ship Cell-/Tissue-Lysates to Zeptosens



Normalization on Total Protein

Lysates are normalized for further processing based on total protein concentrations. Alternatively, samples can be normalized by on-chip protein quantification after spotting.

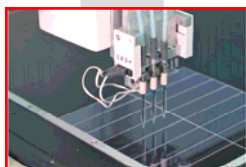
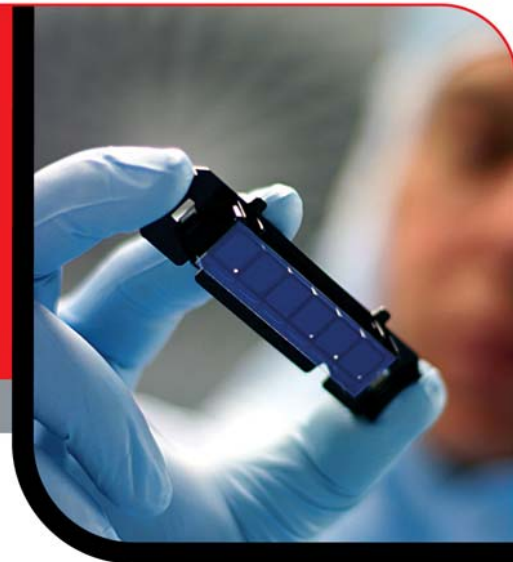


Sample Preparation

Samples are diluted with ZeptoMARK reverse array spotting buffer in a series of four concentrations and then reformatted from 96 to 384 well plates as the source for spotting.

ZeptoMARK Reverse Arrays

Process for Multiplexed Pathway Activity Profiling Services



Spotting of Lysates

Lysates are spotted in duplicates at 4 concentrations on the ZeptoMARK chip coated with our proprietary high adsorbance surface.

Up to 192 different lysates can be positioned on one chip. Since only 400pL of the lysates are required per spot, an almost unlimited number of replica arrays can be prepared from about 100µL of lysate solution.



Blocking the Chip

To minimize non-specific binding of the antibodies, the chip is blocked using ZeptoMARK reverse array blocking buffer in the ZeptoFOG blocking station.

The nebulization of the blocking solution maintains the integrity of the spots and provides an homogenous microarray surface. Up to 40 chips can be treated in one run within 30 minutes.



Assaying

Each of the 6 arrays on a chip is probed with an antibody specifically binding to a signaling protein or its activated form. The fluorescence markers are introduced by application of a labeled anti-species antibody. Only 20 µL of detection antibody (1:250 - 1:10'000 dilution of stock solution) is required per array.



Readout of Arrays

Surface confined fluorophores are measured at green or red excitation wavelengths in a wet state in the ZeptoREADER.

Up to 10 ZeptoCARRIERS, with up to 11'520 lysates can be individually analyzed in an unattended mode overnight.

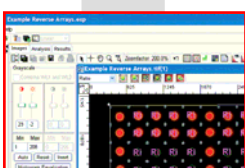
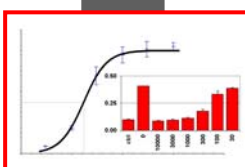


Image and Data Analysis

Signal processing of microarray images taken by the ZeptoREADER is performed with the ZeptoVIEW software.

Data can be exported in a standard format for further analysis.

Report and Raw Data is Sent to Customer



Pathway Activation Profiles

The customer receives a detailed report presenting the results as defined in the beginning of the study. In addition customers get the raw data for in-depth analysis.

Study and results are discussed with specialists from Zeptosens.