

**QUESTIONS**

A Nature of UNIchip<sup>®</sup> .....3

1. How many protein spots are located on the UNIchip<sup>®</sup>? .....3
2. What is the maximum spot number on the current chip layout? .....3
3. Can I get a complete list of all proteins spotted? .....3
4. What protein biochips out of the UNIchip<sup>®</sup>-family are currently available? .....3
5. Do UNIchip<sup>®</sup> protein biochips contain antibodies as well?.....3
6. How many of the spotted proteins are kinases? .....3
7. Where do the proteins come from? .....3
8. Does the UNIClone<sup>®</sup> library contain full-length clones?.....4
9. What is the purity of the proteins on the chip surface?.....4
10. What is the conformation of the proteins on the biochip? .....4
11. Will the proteins be spotted under native conditions onto the biochip? .....4
12. Will the spotted enzymes still be active on the biochip? .....4
13. Do the spots on the chip contain any contaminations due to their expression in *E.coli*?.....4
14. How will the proteins be spotted?.....5
15. What surface is used for the production of UNIchip<sup>®</sup>?.....5
16. What is the average spot diameter?.....5
17. How much protein is contained in each spot? .....5
18. What do you need for spotting customers' antigens and how is it done? .....5
19. What kind of quality controls will be conducted? .....5
20. Which control proteins are printed on the UNIchip<sup>®</sup>?.....5
21. What are the advantages of the selected chip design, especially the "quadruple principle" of spotted proteins? .....6
22. Can I chose a specific content of proteins from the UNIClone<sup>®</sup> library for a customized protein biochip? .....6

B Applications of UNIchip<sup>®</sup> .....6

23. For which kind of applications is the UNIchip<sup>®</sup> primarily designed for? .....6
24. Can I get UNIchip<sup>®</sup> with additional proteins? .....6
25. Can I use the biochip for diagnostic purposes? .....6
26. How do you detect protein binding on the UNIchip<sup>®</sup>? .....6
27. What kind of tags are used for expressed proteins? .....7

28. How long can I store UNlchip®? .....7

29. Why did you put only 384 different human proteins onto the UNlchip®? .....7

C Use of UNlchip® .....7

30. How does the chip readout work and which scanner do you recommend? .....7

31. What is a GAL-File? .....7

32. Which kind of equipment do I need for my own experiments? .....7

33. How can I order UNlchip® products? .....8

34. What is the packaging size and the minimum order quantity?.....8

35. Do you offer antibody characterization with UNlchip® as a service, too? .....8

36. Is there a personal support hotline in case of any experimental problems or questions? .....8

37. How does data acquisition and analysis for the UNlchip® work?.....8

38. Protein productions - Can I get proteins of interest for further experiments?.....9

39. Can I order expression clones out of the UNlclone®-Library? .....9

40. Is there any literature dealing with UNlchip®? .....9

41. Can you name any reference customers for UNlchip®? .....9

## ANSWERS

**A Nature of UNlchip®****1. How many protein spots are located on the UNlchip®?**

At present each UNlchip® AV-400 and UNlchip® AV-VAR contains 2,816 protein spots. Each chip contains 384 different human proteins spotted in quadruplicate (= 1,536 spots), plus up to 320 control protein spots, likewise spotted in quadruplicate (= 1,280 spots, i.e. 10 Control proteins x 2 Concentrations x 2 Duplicates x 32 Subarrays)

**2. What is the maximum spot number on the current chip layout?**

Due to the number and subarray layout (field 1 + 2 contain 16 and field 3 contains 12 subarrays) in combination with the chosen 10x10 grid the maximal spot number is 4,400 (44X100).

**3. Can I get a complete list of all proteins spotted?**

Of course, on request we will send you a complete protein content list of each UNlchip® type marketed as pdf-file.

**4. What protein biochips out of the UNlchip®-family are currently available?**

At present UNlchip® AV-400, UNlchip® AV-VAR EP, AV-VAR MP, and UNlchip® AV-VAR IP are available.

**5. Do UNlchip® protein biochips contain antibodies as well?**

Each biochip contains 5 antibodies of different species (human, mouse, rat, goat and rabbit) utilized as positive controls for the binding of the selected secondary antibody. Beyond these control proteins no antibodies are printed, UNlchip® is not an antibody array.

**6. How many of the spotted proteins are kinases?**

The proteins represent a cross-section of multiple gene families including pharmaceutically relevant protein classes such as kinases, membrane-associated proteins, cell-signalling proteins and metabolic proteins. As UNlchip® product series is primarily designed for antibody validation and not for kinase profiling, covered kinases are meant for detection of potential off-target activities.

**7. Where do the proteins come from?**

All proteins printed on the microarrays are derived from the UNlclone® technology of Protagen AG. With UNlclone®, different well characterized human expression libraries (= UNlclone®-sets) have been generated, which currently contain more than 10,000

non-redundant recombinant human clones. One library was generated from fetal brain, another from human T-helper cells.

Each human open reading frame (ORF) is expressed as an N-terminal His-tag fusion protein using *Escherichia coli*.

**8. Does the UN1clone® library contain full-length clones?**

Approximately 60% of all clones out of the UN1clone® library are full-length.

**9. What is the purity of the proteins on the chip surface?**

The purity of the spotted proteins is above 80%. Quality analysis has been done by 1D-PAGE.

**10. What is the conformation of the proteins on the biochip?**

The purification of the proteins is done under denaturing conditions. Subsequently proteins are spotted onto the nitrocellulose-coated glass slide using a urea buffer.

However, experiments give strong evidence that the majority of the proteins regain their natural conformations. This was demonstrated using GFP, which showed full functionality after purification under exactly the same conditions. Likewise we found positive interactions between the spotted proteins and therapeutic antibodies as well as for antibodies, which failed to detect their antigen in Western Blot. Therefore we call the conditions on UN1chip® “renaturing”.

**11. Will the proteins be spotted under native conditions onto the biochip?**

No, standard UN1chip® proteins will be spotted under denaturing conditions (see also question above). However, customer-supplied proteins can be printed as supplied by the customer including native conditions.

**12. Will the spotted enzymes still be active on the biochip?**

Experiments with GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) showed enzymatic on-chip activity. However, functional enzymatic activity is not part of the UN1chip® specification.

**13. Do the spots on the chip contain any contaminations due to their expression in *E.coli*?**

Based on a purity of more than 80% (see question 9) only a minor remaining quantity of *E. coli* proteins can be contained on the biochip. These small *E. coli* protein quantities have never interfered/biased any of our biochip experiments to date.

**14. How will the proteins be spotted?**

Proteins will be spotted by contact spotter QArrayMax (Genetix), a sophisticated, high-throughput system for microarraying. For each spot a volume of approx. 2 nl is applied.

**15. What surface is used for the production of UNlchip®?**

To obtain high quality, i.e. high sensitivity, high reproducibility and a long shelf life, we use nitrocellulose-coated (NC) glass slides.

**16. What is the average spot diameter?**

The spot diameter ranges between 100 and 150 µm.

**17. How much protein is contained in each spot?**

Typical protein concentrations range 1 - 5 fmol per spot.

**18. What do you need for spotting customers' antigens and how is it done?**

For the spotting of antigens onto UNlchip® the customer has to provide 400 µl of each antigen sample which should be in a concentration of at least > 0.5 mg/ml. The antigen samples should be contained in PBS buffer or 6M Urea buffer. The purity of the antigen depends upon the purpose of the experiment.

**19. What kind of quality controls will be conducted?**

Protagen AG established rigorous production and quality control procedures.

*Pre-printing quality control*

Prior to production, the spotting robot is tested and adjusted to production specifications. The quality and performance of pins is critical and all pins are tested and calibrated. To avoid cross-contaminations specific washing procedures were developed for the printing process.

*Post-printing quality control*

After production each microarray is visually inspected for obvious defects which could interfere with experimental results. To control the quality of the printing process, each production lot is probed with an anti-RGSHis antibody. Since the purified human proteins contain RGSHis tags, incubating the UNlchip® with an anti-RGSHis antibody allows identification of irregular spot morphology or missing spots.

**20. Which control proteins are printed on the UNlchip®?**

Altogether we have included 10 different control proteins, i.e. 5 control proteins for species-specific secondary antibodies (human, mouse, rabbit, goat and rat), two non-human controls for verification of background level (Horse Radish Peroxidase (HRP) and Lysozym), and GAPDH, HSP-90 and Stathmin as human process controls.

**21. What are the advantages of the selected chip design, especially the “quadruple principle” of spotted proteins?**

The selected chip and subarray layout leads to a higher validity and reproducibility of the results of each UNlchip®. Due to the distribution of different reading point across the complete chip surface in subarrays, intra-chip variance can be quantified. The “quadruple principle”, i.e. four different data for each protein, increases the statistical relevance of the results significantly. In particular, the median intensity of binding signals can be calculated which yields a statistically more robust value than the mean.

**22. Can I chose a specific content of proteins from the UNlclone® library for a customized protein biochip?**

Protagen AG offers different types and versions of the UNlchip® family. Especially with the UNlchip® AV-VAR, a highly specialized tool is available as its 400 proteins are focused on different GO classes. However, a fully customized protein biochip is only available on request in a minimum order quantity, as the complexity and effort will be significantly higher in comparison to our standard products. Please contact us for further information or inquiries on this topic.

**B Applications of UNlchip®****23. For which kind of applications is the UNlchip® primarily designed for?**

The members of the UNlchip® family of Protagen AG are high-density protein microarrays primarily designed for antibody characterization. In principle, other applications in the area of protein-protein interactions are possible like serum, kinase or protease profiling or protein-small molecule interactions. We will be happy to discuss application opportunities based on the most recent experience with our customers.

**24. Can I get UNlchip® with additional proteins?**

Technically it is no problem to put additional customer specific protein onto the UNlchip®. Please contact us for further information or inquiries on this topic.

**25. Can I use the biochip for diagnostic purposes?**

NO, UNlchip® protein biochips are designed for research purposes only. A research application is not intended for in vitro diagnostic purposes or clinical use, but is intended solely for use in the research setting, for example pharmaceutical development or university.

**26. How do you detect protein binding on the UNlchip®?**

Antibodies that bind to UNlchip® proteins are detected by incubating arrays with a fluorescent labeled secondary antibody. Since the protein identity of each spot on the

microarray is known, proteins that interact specifically with customer-provided antibodies can be quickly identified.

We recommend the use of Cy3 labeled secondary antibodies, which are broadly commercially available. We have listed additional compatible labeled secondary antibodies in the instruction manual.

**27. What kind of tags are used for expressed proteins?**

Each human open reading frame (ORF) is expressed as an N-terminal RGS 6x His-tag fusion protein.

**28. How long can I store UNlchip®?**

UNlchip® protein biochips are shipped and stored at +4 °C. Under these conditions they can be stored at least 4 months.

**29. Why did you put only 384 different human proteins onto the UNlchip®?**

According to our experimental experiences this number of different proteins is sufficient, to determine the degree of off-target activities and rank antibodies (or other protein binders) according to specificity. The relative amount of off-target activity remains relative constant even when using higher numbers of proteins.

## C Use of UNlchip®

**30. How does the chip readout work and which scanner do you recommend?**

For the detection of positive protein interactions the microarray is scanned using a suitable fluorescent microarray scanner. We use a ProScanArray HT (PerkinElmer Life and Analytical Sciences, Wellesley, USA). The scanner specifications required to image the UNlchip® and compatible scanners are listed in the instruction manual.

**31. What is a GAL-File?**

The GenePix Array List file is a text file essential for data acquisition by the software and defines spot locations and identities of all protein spots on the array. In addition, this file is necessary for creating a grid on the UNlchip®.

**32. Which kind of equipment do I need for my own experiments?**

To use the premium version of the different UNlchip® types, you only need a suitable fluorescent microarray scanner (see question 30). When ordering the standard version of UNlchip® (field 3 = customer field is empty) you should be equipped with a microarray spotter, to print your own antigens if this is desired.

**33. How can I order UNlchip® products?**

We are happy to send you our latest price list and a quotation. You can directly order by fax +49 (0) 231-9742-6301 or by mail: [unichip@protagen.de](mailto:unichip@protagen.de)

**34. What is the packaging size and the minimum order quantity?**

Our standard UNlchip® package contains 5 UNlchip® Biochips and an instruction manual. However, there is no minimum order quantity.

**35. Do you offer antibody characterization with UNlchip® as a service, too?**

Yes, Protagen AG offers a comprehensive antibody characterization and specification service. This full service package includes study design, protein biochip production, scanning, and analysis service and will provide you with a detailed report of all results.

This means an easy and convenient access to the UNlchip® technology with:

- High quality content (human proteins)
- guaranteed output and high quality results
- Extensive experience in protein biochip technology plus state-of-the-art lab instrumentation
- No large upfront investments or allocation of internal resources required
- Fast and efficient processing to receive quick and reliable results
- High quality and reproducibility of data due to standardized processes including numerous quality controls

Please do not hesitate to contact us for further information or request our sales team for special offers and conditions.

**36. Is there a personal support hotline in case of any experimental problems or questions?**

Yes, in case of any questions or if you need technical assistance please call +1-888-879-1445 (USA) or +49-231-9742-6300 (Europe). We will connect you directly with an experienced specialist who will answer your questions.

**37. How does data acquisition and analysis for the UNlchip® work?**

To acquire data from the saved images, you will need appropriate microarray data acquisition software. We recommend microarray image analysis software such as GenePix® Pro (Molecular Devices Corporation), GeneSpotter (MicroDiscovery GmbH) or ScanArray® Software (PerkinElmer Inc.). Normally this kind of software is bundled with the various microarray scanners.

**38. Protein productions - Can I get proteins of interest for further experiments?**

Yes, the protein of interest can be cloned and expressed in *E.coli*. We can provide protein production up to low mg amounts. Please contact us for special offers and conditions.

**39. Can I order expression clones out of the UNIclone<sup>®</sup>-Library?**

We are unable to offer you single clones out of the UNIclone<sup>®</sup> library as they are property of Protagen AG.

**40. Is there any literature dealing with UNIchip<sup>®</sup>?**

We are happy to send you a comprehensive list with all relevant publications.

**41. Can you name any reference customers for UNIchip<sup>®</sup>?**

Major reference customers for UNIchip<sup>®</sup> products and services are a business unit of MorphoSys AG and a unit of Novartis AG. Please call us and we are happy to bring you in contact with the persons responsible.

**For more information or inquiries please contact:**

<p><b>Protagen AG</b> Protein Biochips Otto-Hahn-Str. 15 44227 Dortmund, Germany</p> <p>Phone: +49 231 9742 6300 Fax: +49 231 9742 6301 E-mail: <a href="mailto:unichip@protagen.de">unichip@protagen.de</a></p>
--