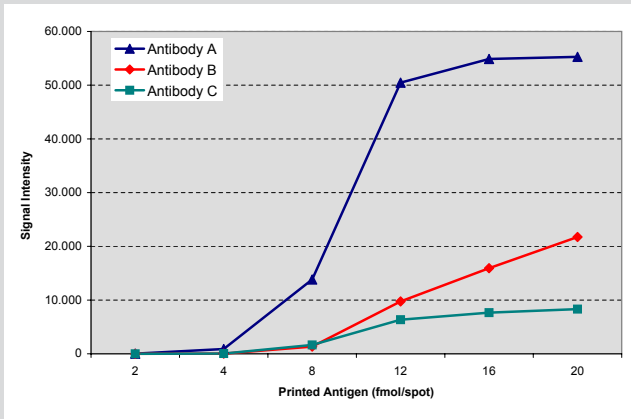


Application Example: Antibody selection for further development

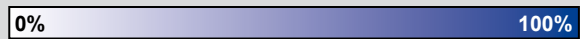


Linearity, sensitivity and dynamic range of three different antibodies targeted against the same antigen.

The antigen was spotted onto UNichip® AV-400 in quadruplicate in different concentrations. Binding of the antibodies was visualized using a fluorophor-labelled secondary antibody in a standard DNA-microarray scanner. Antibody A shows the best overall performance.

Antigen/ OTA	ANTIBODIES		
	A	B	C
Antigen	100	100	100
OTA #1	0	10	43
OTA #2	0	10	96
OTA #3	0	7	82
OTA #4	0	7	50
OTA #5	0	9	77
OTA #6	39	0	0
OTA #7	13	0	0
OTA #8	0	8	149

Quantitative antibody fingerprint based on 400 different human proteins.



Color code: Maximum antigen signal = 100%  
All specifically reacting proteins can be clearly assigned. Antibody A showed only two off-target activities (=OTAs), whereas antibody B and C revealed 6 OTAs with other proteins. For antibody C one of these (#8) was even higher than the signal intensity with the target antigen.

Goal

An industry leading biotechnology company has developed several different antibodies against a serine protease inhibitor. At this stage an antibody has to be selected for further development.

Solution provided with UNichip® AV-400

Protagen AG has printed the customer's target antigen in different concentrations onto its UNichip® AV-400. The three different antibodies were ranked by comparing the relative sensitivity and affinity of the binding curves. In addition a quantitative fingerprint was obtained for each antibody, showing highly similar binding profile for antibodies B and C and a distinct profile for antibody A.

Results & Benefits

The use of UNichip® protein biochips allowed selection of the antibody which showed superior overall performance and only minor off-target activities in the quantitative fingerprint within a very short time.

„Antibody A“ was selected for further development. This decision was confirmed by other experiments and performance criteria made by the customer. The UNichip® AV-400 enabled the customer to make a much better preliminary selection/ranking of antibodies for further development, thus lowering development time and costs.

References

Determination and Validation of Off-Target Activities of anti-CD44 Variant 6 Antibodies employing Protein Biochips and Tissue-Microarrays; Lueking A., Beator J., Patz E., Mueller S., Mehes G., Amersdorfer P.; Biotechniques 2008, in press.  
Off-target activity of TNF-alpha inhibitors characterized by protein biochips; Feyen O., Lueking A., Kowald A., Stephan C., Meyer H.E., Göbel U., Niehues T.; Analytical and Bioanalytical Chemistry, 2008 Mar 16.  
Application Note 201/2006 - Validation of TNFα antibodies with UNichip® AV-400; Lueking et al., Jan 2006.

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UNichip® Protein Biochips

Quantitative binding profiles for antibody characterization

Powerful Protein Biochips

Scientists involved in antibody development and characterization need to compare and evaluate many candidates. They will save time on downstream assays by incorporating the UNichip® protein biochip into the antibody development work flow. UNichip® protein biochips are available both, as a full service package and as a stand alone product.

The UNichip® protein biochip enables early selection of the best antibody by providing quantitative antibody fingerprints. With these unique binding profiles different antibodies can easily be ranked according to their degree of specificity. The selection of truly monospecific antibodies is a novel method for improved risk assessment of drug candidates (see references). It reduces the risk of failure due to unexpected off-target activities and results in cost savings due to a shorter time to market.

As part of a proprietary biochip layout customers' antigen(s) can be printed onto the UNichip® and relative signal intensities of the 400 human proteins can be compared to the target antigen(s).

Discuss your specific antibody development project with Protagen scientists to exploit the full scope of the groundbreaking protein array technology.



**Powerful applications**

With access to high value human protein content and the ability to generate reproducible and quantitative fingerprints, UNIchip® protein biochips enable a broad range of applications:

- ▶ *Antibody Development*
- ▶ *Antibody Production Monitoring*
- ▶ *Quality Control of Antibody Production*
- ▶ *ELISA/Assay Development*

FEATURES	BENEFITS
Quantitative binding profiles (= fingerprints)	<ul style="list-style-type: none"> <li>▶ Ranks antibodies by specificity</li> <li>▶ Reduces risk of off-target activity</li> <li>▶ Saves costs by elimination of unspecific antibodies</li> </ul>
Cognate antigen(s) printed on the biochip	<ul style="list-style-type: none"> <li>▶ Enables unique affinity ranking of antibodies</li> <li>▶ Serves as positive control for the tested antibody</li> </ul>
400 recombinant human and control proteins	<ul style="list-style-type: none"> <li>▶ Statistically significant number of different targets for specificity analysis</li> </ul>
Different protein contents available	<ul style="list-style-type: none"> <li>▶ Project-specific choice of ideal protein subset</li> </ul>
Each protein printed in quadruplicates (2 x duplicates)	<ul style="list-style-type: none"> <li>▶ High confidence in results</li> </ul>
All recombinant proteins are MALDI-MS verified	<ul style="list-style-type: none"> <li>▶ Full confidence in identified off-target activities</li> </ul>
Free open access software tool for data analysis	<ul style="list-style-type: none"> <li>▶ Ensures full transparency of results</li> <li>▶ Easy data export to other software packages</li> </ul>
Full service package or ready-to-use biochips	<ul style="list-style-type: none"> <li>▶ Easy technology assessment without capital investment</li> <li>▶ No allocation or training of internal personnel required</li> <li>▶ In-house use for routine users after technology evaluation</li> </ul>

**Full Service Package**

Take advantage of our comprehensive UNIchip®-based antibody analysis service. This full service package includes study design, protein biochip production, chip scanning, and analysis. A detailed report with all results is also included.

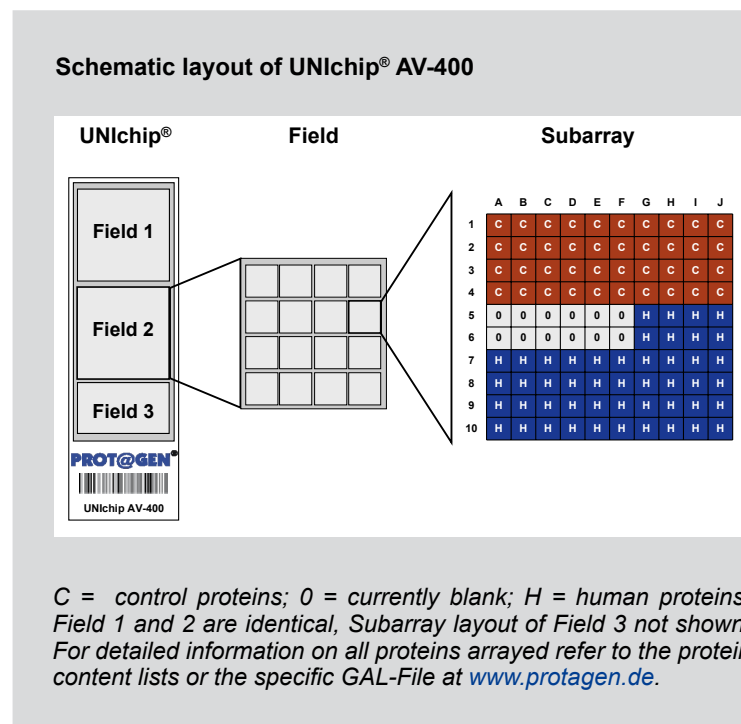
**Powerful Analysis Software**

UNIchip® data can be analysed with a cost-free open access software tool. Raw data are analysed within minutes to generate standardized binding curves and quantitative fingerprints with protein names for all off-target activities. The UNIchip data analysis tool can be integrated into Microsoft® Excel and gives full transparency of all results. Data and graphics can be easily exported for further reporting or calculation purposes.

**Unique design of the UNIchip®**

The UNIchip® design has two identical fields on a nitrocellulose-coated glass slide. The proteins within each field are printed in 16 subarrays which include control (C) and recombinant human proteins (H). Each recombinant human protein is printed in duplicate within each single field and thus is present in quadruplicates (4x) on each UNIchip®. This patented chip layout leads to higher validity and statistical relevance of the results.

In field 3 the customer's antigen(s) or other proteins can be printed in a serial dilution. In a unique way this enables a comparative sensitivity and affinity ranking of different antibodies against the same antigen(s) in addition to the quantitative fingerprint.



**Specifications**

<b>Dimensions:</b>	1 inch x 3 inch (25.4 mm x 76.2 mm)
<b>Material:</b>	glass slide coated with nitrocellulose membrane
<b>Membrane dimensions:</b>	20 mm x 51 mm
<b>Average amount of protein per spot:</b>	1 -5 fmol per spot (50 - 250 pg/spot)
<b>Working volume:</b>	200 - 500 µl
<b>Range of antibody concentration</b>	0.2 - 10 µg/ml typically
<b>Number of human proteins:</b>	4 sets à 384 (spotted in quadruplicate) plus control proteins



**Basic or Premium Version**

In the basic version the individual antigen can be printed onto field 3 by the customer. Alternatively, the protein biochips can be used directly for the generation of antibody fingerprints without printing an antigen. In the premium version the customer provides the antigen (up to 8 different antigens possible) to Protagen AG, where it will be printed onto field 3 of the protein biochip in a defined serial dilution.

**Products and Services available**

Product	Size	Cat. No. Basic	Cat. No. Premium
UNIchip® AV-400	5 Biochips	1401-2000	1401-2010
UNIchip® AV-VAR EP	5 Biochips	1401-3000	1401-3010
UNIchip® AV-VAR MP	5 Biochips	1401-3002	1401-3012
UNIchip® AV-VAR IP	5 Biochips	1401-3001	1401-3011

Service	Size	Cat. No.	Note
UNIchip® Service	per Antibody	1401-3030	UNIchip® selectable

**Note:** The UNIchip® AV-400 contains a cross section of all cellular proteins, whereas the AV-VAR series focuses on different Gene Ontology classes (EP = Extracellular Proteins; MP = Membrane Proteins; IP = Intracellular Proteins).

**UNIchip® fingerprint predicts antibody performance in immunohistochemistry**

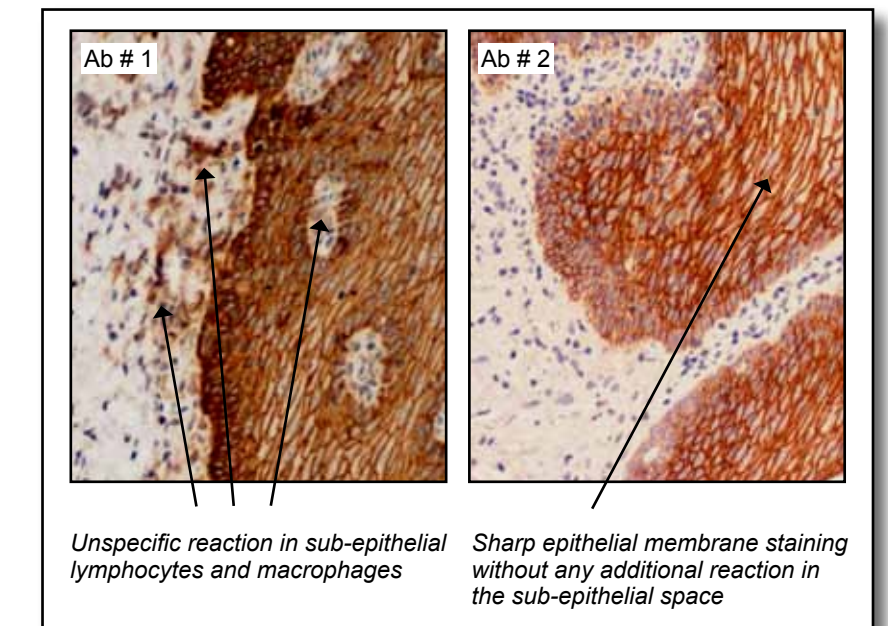
Two high affinity monoclonal antibodies directed against CD44v6 were analysed using UNIchip® AV-400. Binding to the UNIchip® proteins was visualised using a fluorophor-labelled secondary anti-human IgG antibody. The number of off-target activities was determined using the UNIchip® data analysis tool. Immunohistochemical analysis of normal esophagus tissue was performed using optimized antibody concentrations for each antibody (2 ng/ml for # 1, and 6.7 ng/ml for # 2). Antibody # 2 is highly specific both on the UNIchip® and in the immunohistochemical (IHC) analysis.

The antibodies were used at low ng/ml concentrations both for UNIchip® and IHC analysis. Note that the specificity ranking of the antibodies is independent of the concentration used for UNIchip® analysis.

Concentration of antibody	# of off-target activities	
	Ab # 1	Ab # 2
2 ng/ml	126	1
6.7 ng/ml	206	2

IHC Data derive from a collaborative study with Oridis Biomed GmbH, TISSO-MICST™ expert partner for tissue based research ([www.oridis-biomed.com](http://www.oridis-biomed.com)).

Data underpin the potential of UNIchip® protein biochips to identify best candidate(s) for FDA-required immunohistochemistry analyses.



**Select the service option for:**

- *Quick and reliable 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*



**Additional analytical services for antibody characterization/lot-release testing:**  
(GMP-compliant on request)

- ▶ Identification of unknown antigens/targets
- ▶ Structural characterisation
- ▶ Physicochemical properties
- ▶ Process- or product-related impurities
- ▶ Peptide mapping/Isotype-determination