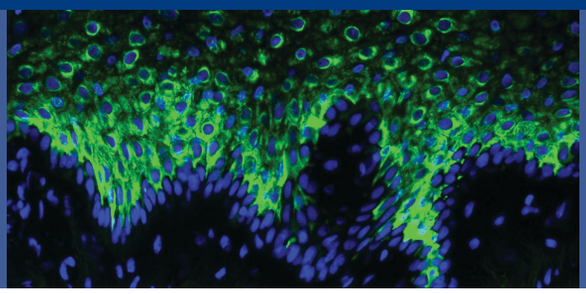
CONTENT

- Pilot project for antibody validation using PEPperMAP® peptide mapping technology
- Selectivity and specificity of keratin 10 & 18 antibodies confirmed
- New information on epitope specificity gained



Validation of keratin antibodies for western blots by peptide mapping

Novel antibody profiling delivers precise data on epitope specificity & cross-reactivity

BACKGROUND & OBJECTIVES

Keratins are a large family of essential structural proteins that are expressed in a highly tissue- and differentiation-specific manner and organizational pattern that is typical for the different epithelial tissues. Anomalies in keratin expression or function are often associated with disease: More than 60 different disorders including liver and skin diseases, cancer or autoimmune reactions against keratins have been described. In addition, keratins are important diagnostic and prognostic markers in cancer and might even play an active role in tumorgenesis [reviewed by Karantza, 2011][1].

As central epithelial markers in health and disease, keratins are under intense study in basic and applied cell biology research. In order to correctly identify keratin subtypes and analyze their expression patterns by western blot, scientists need reliable antibodies that generate reproducible data.

PROGEN's keratin antibodies are market-proven and their performance in immunohistochemistry is well documented. In order to provide additional validation data for western blot, PROGEN has launched a program that utilizes the peptide mapping service PEPperMAP® of its collaborator PEPperPRINT to generate dependable data on selectivity and specificity of the keratin antibodies. The array allows testing for cross-reactivity and epitope mapping of purified antibodies and is also suitable for testing auto-antibodies in patient serum samples. The keratins selected for this validation have been described in the literature as targets of human auto-antibodies in different diseases.

This application note describes the evaluation of PROGEN's mouse monoclonal antibodies against keratin 10 (Cat. No. 11414, clone DE-K10) and keratin 18 (Cat. No. 61028, clone Ks18.04), that have been linked to Chronic Lyme Arthritis and Chronic Obstructive Pulmonary Disease (COPD), respectively[2,3]. On western blot, the keratin 10 and keratin 18 antibodies specifically recognize their respective antigens in HEK293 and MCF-7 cell lysates (see figure 1). In immunohistochemistry, both antibodies show a distinct pattern, confirmed by independent results with alternative antibodies against the corresponding antigens (data not shown).

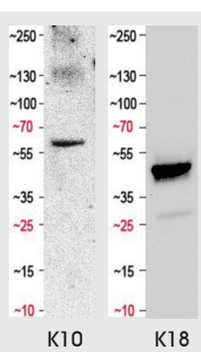


Figure 1| *Western blots using PROGEN's K10 and K18 mouse monoclonal antibodies on cell lysates (K10: 5µg HEK293, K18: 5µg MCF-7 lysate). Each antibody generates a distinct band on western blot.*

[1] Karantza V, 2011, Oncogene 30:127–138
[2] Ghosh S et al., 2006, J Immunol 177:2486–2494
[3] Xiong Y et al., 2017, Arch Med Res 48:79–87

PROGEN passion for research

METHODS

The workflow followed a two-step approach:

1. Identification of peptides / epitopes that are bound by the target antibody

Peptide microarrays of the target antigen were generated by translating antigen sequences of relevant keratins (8, 10, 14, 18, and 19) into 15 aminoacid (aa) peptide fragments at single amino acid resolution, i.e. with a peptide-peptide overlap of 14 aa. Incubation of this peptide microarray with PROGEN's monoclonal mouse keratin antibodies K10 or K18 and a fluorescently labeled secondary antibody gave rise to a spot pattern that indicated immunoreactions of the target antibody with matching peptide fragments.

2. Discovery of common motifs in the top peptide hits

For each tested antibody, a microarray scan was performed with increasing amounts of the antibody in order to find the best signal to noise condition for each antibody. An intensity plot revealed a stretch of adjacent peptides with a motif that represents the linear epitope of the given antibody. Generally, higher signal intensities at low concentrations indicate a high affinity of the antibody while high signal above background indicate a high specificity of the antibody.

RESULTS

Keratin 10 antibody

The mouse monoclonal keratin 10 antibody (clone DE-K10, cell culture supernatant) was applied at a 1:10 dilution for the peptide microarray incubation. The antibody reacted exclusively with the "GGGXFX" consensus motif in three different keratin type I cytoskeletal 10 fragments. No signal was found for peptides from keratin 8, 14, 18, or 19 (see figure 2). These data demonstrate clearly that the keratin 10 antibody is highly specific for keratin 10 and reveals no cross-reactivity towards the other tested keratin peptides.

Keratin 18 antibody

The mouse monoclonal keratin 18 antibody (Ks18.04) was analyzed with the peptide microarray at a concentration of 10 µg/mL. It was highly specific for keratin 18 and bound to the peptide "LETEIEALKEELLF". A similar sequence present in keratin 19 caused a weak cross-reaction at background level (see figure 3). These data reveal a strong specificity of the keratin 18 antibody (Ks18.04) for this epitope.

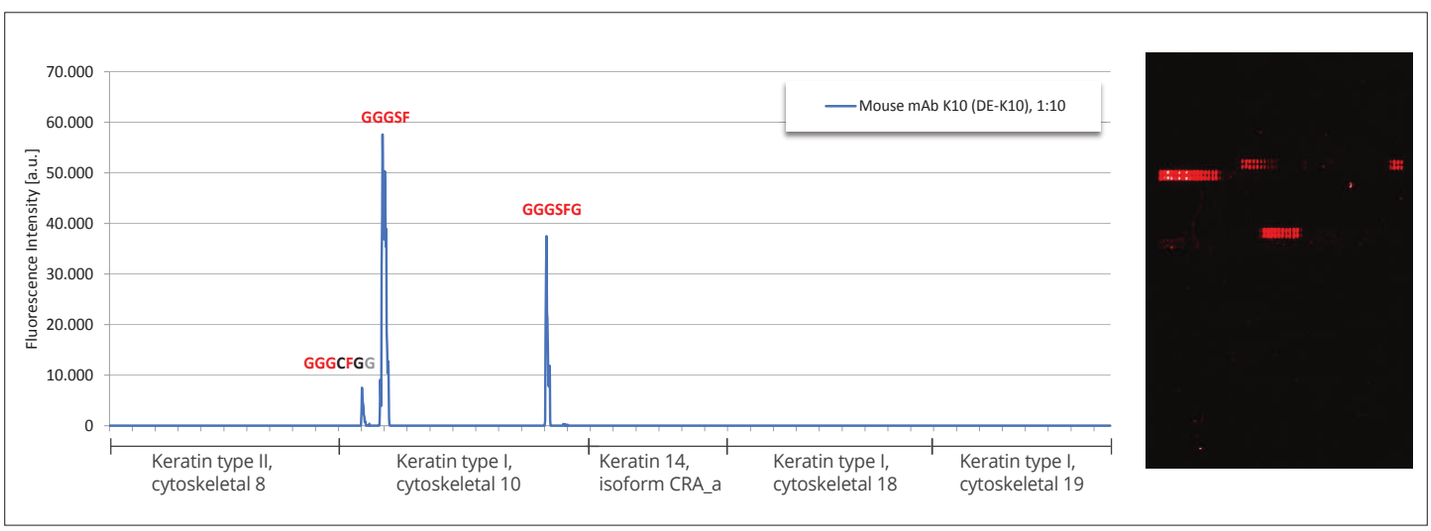


Figure 2| *Microarray of overlapping peptide fragments of keratins 8, 10, 14, 18, and 19, incubated with the K10 (DE-K10) antibody. Right panel: Immunofluorescent microarray spots visualize specific immunoreactions of the K10 antibody with matching peptide fragments. Left panel: Intensity plot with distinct binding peaks at the consensus motif „GGGXFX" of three peptide fragments.*

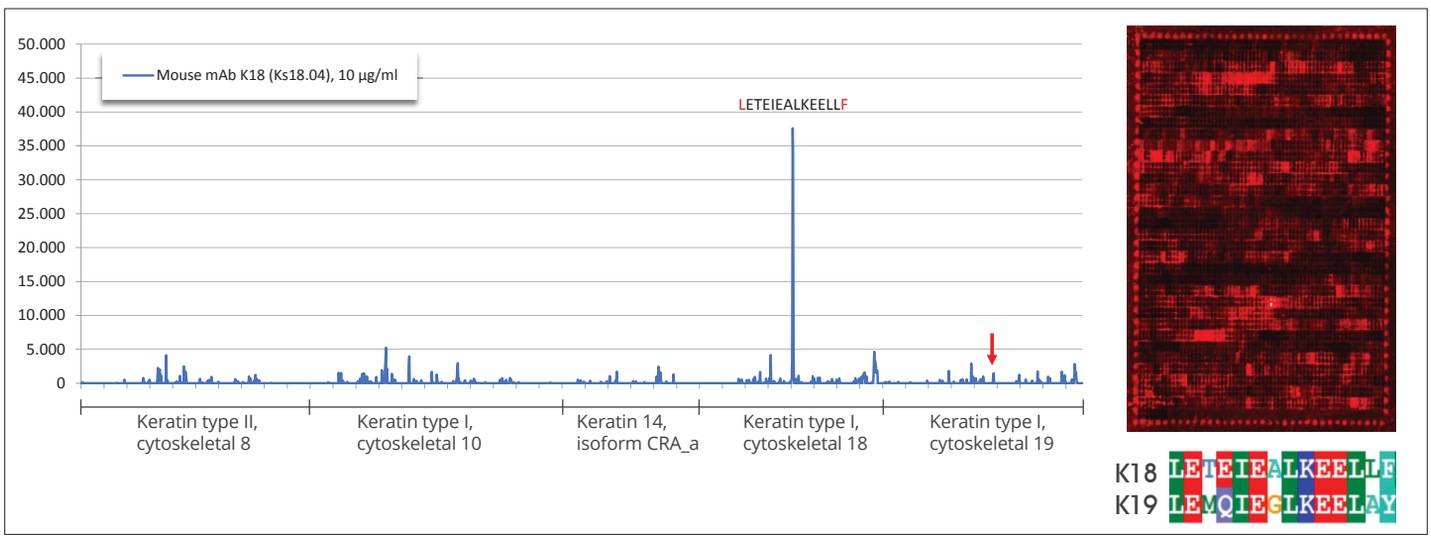


Figure 3| *Microarray of overlapping peptide fragments of keratin 8, 10, 14, 18, and 19, incubated with the K18 (Ks18.04) antibody. Right panel: Immunofluorescent microarray spots visualize specific immunoreactions of the K18 antibody with matching peptide fragments. Left panel: Intensity plot with a pronounced binding peak at the consensus motif „LETEIEALKEELLF" of the keratin 18 peptide fragment containing the epitope and background signals for other peptides (red arrow marks binding to similar consensus motif in keratin 19. Bottom right panel: Overlapping binding motifs in keratin 18 and keratin 19.*

DISCUSSION & CONCLUSION

PROGEN tested the peptide microarray analysis as a novel approach to validate its keratin antibodies for western blot. The PEPperPRINT array platform enables the analysis of peptides covering up to 20 proteins (depending on protein size and sequence similarity) on the same array with a single amino acid resolution. The identification of specific binding epitopes using this technique has confirmed the high quality and reliability of the employed keratin antibodies and has generated valuable information to researchers who may now use these antibodies with even more confidence. Like most validation techniques, this approach is not applicable to all possible binding modes of antibodies. For example, no signal in this array could indicate a different type of epitope like discontinuous peptide sequences, unknown post-translational modifications that the antibody might bind in the native target, or a peculiar secondary structure of the peptide that is not observed in the full-length protein.

In a first pilot project, PROGEN's mouse monoclonal antibodies for keratin 10 and 18 were analyzed and the generated data have not only confirmed the high degree of selectivity and specificity of these antibodies but have also revealed new information on epitope specificity. Given this positive outcome and the seamless cooperation with PEPperPRINT, PROGEN plans to implement the independent validation by peptide mapping for the majority of its antibodies.

This program is yet another module in PROGEN's multiple efforts – which include external quality control for every new lot produced, extensive western blot data and positive western blot controls – to provide premium antibodies that meet the increasing demand for more reliable and validated immunochemicals to enable clear and reproducible scientific results.

SELECT FROM OUR PORTFOLIO OF

TISSUE-SPECIFIC
KERATIN ANTIBODIES

2018 AWARDS
CiteAb
HIGHLY COMMENDED
PROGEN
ANTIBODY VALIDATION INITIATIVE AWARD

CONTACT US

info@progen.com
www.progen.com

SHOP ONLINE

www.progen.com

PROGEN
passion for research

PROGEN Biotechnik GmbH
Maaßstr. 30 • 69123 Heidelberg, Germany
T +49 6221 8278-0 • F +49 6221 8278-24