## Validating Antibodies for IHC: a Complex Technology

The Antibody Society Webcast series – Antibody Validation #6

Jim Trimmer Department of Physiology & Membrane Biology University of California, Davis School of Medicine jtrimmer@ucdavis.edu

ANTI BODY SOCI .ETY

#### Antibodies are "Fit-for-Purpose" and not "All-Purpose" Reagents

It follows directly that antibodies need to be validated in the **specific application** and under the **specific sample preparation conditions** in which they will be used

Comprehensive Screening of many Antibodies Supports Their Efficacy as "Fit-for-Purpose" Reagents

Analysis of 96 ELISA-positive antibodies by:

ICC: immunocytochemistry against transfected cells overexpressing target protein

WB: Western blot against brain sample

IHC: immunohistochemistry against brain sections





ETY

## General IHC Considerations-I

 Validation in native tissue is crucial: cell-specific PTMs and interacting proteins can fundamentally impact antibody binding, and these may not be present in other preparations (e.g., overexpression) or other applications (e.g., Western blots)

### Orthogonal Antibody Validation

Compare mRNA and protein expression patterns in a tissue that contains cells with differing levels of expression of antigen

In brain neurons, mRNA in cell body; protein could be localized there, but could also be in extensive processes (dendrites and/or axons)



ANTI

BODY

SOCI

ETY



#### Genetic Validation



Kv4.2 Kv2.1 Menegola & Trimmer, J Neurosci 26:12137, 2006









ANTI BODY SOCI . ETY

Burkhalter, Conchar, Mellor & Nerbonne, J Neurosci 26:12274, 2006

2/3

5

6

Misonou et al., J Comp Neurol 496:289, 2006

## General IHC Considerations-II

• Sample preparation conditions for IHC: fundamentally impact antigen structure, and chemical properties of its amino acid side chains, as well as antibody penetration into the tissue itself





#### Post-Hoc Antigen Retrieval Can Expose Epitopes Occluded in Fixed Tissue

No Ag retrieval

With Ag retrieval



ANTI BODY SOCI . ETY

Perfusion-fixed with 4% FA, pH 7.4 (standard fixation for basic research IHC)

Sections in bottom row treated with 0.1 M sodium citrate, pH 8.5, for 30 min at 80 °C

Protocol from Jiao et al., J Neurosci Methods 93:149, 1999

#### Post-Hoc Antigen Retrieval Can Expose Epitopes Occluded in Fixed Tissue



Perfusion-fixed with 4% FA, pH 7.4 (standard fixation for basic research IHC)

Sections in right column treated with pepsin

Lorincz and Nusser. Specificity of Immunoreactions: The Importance of Testing Specificity in Each Method. J Neurosci 28:9083, 2008

> ANTI BODY SOCI . ETY

## Conclusions

It is critical to focus on validating antibodies for use in specific applications and using specific sample preparation conditions (and then reporting these conditions transparently so that your work can be reproduced and extended)

Sample preparation and especially fixation has substantial consequences on efficacy and specificity of antibody labeling in IHC

Orthogonal and genetic validation approaches are especially useful for IHC but need to be combined with the above considerations to be effective



## Maybe Routine, but non-trivial – Validation in Practice #1

The Antibody Society Webcast series – Antibody Validation #6

Aldrin Gomes James Trimmer University of California, Davis University of California, Davis, School of Medicine

> ANTI BODY SOCI . ETY

#### Next Webcast in Antibody Validation: a 9-part series

- 1. Andreas Pluckthun
- 2. Glenn Begley Cecilia Williams
- 3. Jan Voskuil
  - Andy Chalmers
- 4. Anita Bardowski Jan Voskuil
- 5. Giovanna Roncador:
- 6. Aldrin Gomes Jim Trimmer
- 7. Travis Hardcastle Alejandra Solache
- 8. Mike Taussig Fridjhof Lund-Johansen
- 9. Andrew Bradbury Andreas Pluckthun

- : The different antibody formats
- : Antibodies and the reproducibility crisis in biological science
- : The Erß story is your antibody like this?
- : Beware the supplier OEM
- : Finding antibodies in the Antibody Databases
- : Which antibody are you looking for? The RRID
- Points to note on the supplier datasheets
- : Correct positive and negative controls in validation
- Western blots: not as easy as it looks
- : IHC issues: in brain sciences
- : Cell KO technology
  - Validating Antibodies with KO technology
  - Validating antibodies using array technologies

ANTI BODY SOCI

- : Mass spectroscopy for mass validation
- : Why publish sequences?
- : What are the coming alternatives ?

# Validation of Commercial tool Antibodies

#### Maybe Routine, but non-trivial – Validation in Practice #1 The Antibody Society Webcast series – Antibody Validation #6

Presented by Aldrin Gomes and James Trimmer

Produced and Directed by Simon L. Goodman Production Manager Fran Breden Writen by Simon Goodman

https://www.antibodysociety.org/

ANTI BODY SOCI . ETY

### Validation of Commercial Tool Antibodies

An Antibody Society Webcast series

Administrative Support: Dr. Fran Breden and Dr. Mini Muralidharan

Executive Director: Dr. Jan Reichert

This series would be impossible without the generous financial support of our Corporate Sponsors:



https://www.antibodysociety.org/