Validation of Commercial tool Antibodies

The Antibody Society Webcast series – Antibody Validation #5

Fit-for-purpose Positive and Negative controls

Simon L. Goodman
Science and Technology Program Manager
The Antibody Society

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Antibody Validation: a 9-part series

1.	Andreas Pluckthun	:	The different antibody formats
2.	Glenn Begley	:	Antibodies and the reproducibility crisis in biological science
	Cecilia Williams	:	The Erß story – is your antibody like this?
3.	Jan Voskuil	:	Beware the supplier OEM
	Andy Chalmers	:	Finding antibodies in the Antibody Databases
4.	Anita Bardowski	:	Which antibody are you looking for? The RRID
	Jan Voskuil	:	Points to note on the supplier datasheets
5.	**Giovanna Roncador:**	**:**	**Correct positive and negative controls in validation**
6.	Aldrin Gomes	:	Standard technology: "even" Western blots are non-trivial
	Jim Trimmer	:	IHC issues in brain sciences
7.	Travis Hardcastle	:	Cell KO technology
	Alejandra Solache	:	Validating Antibodies with KO technology
8.	Mike Taussig	:	Validating antibodies using array technologies
	Fridjhof Lund-Johansen	:	Mass spectroscopy for mass validation
9.	Andrew Bradbury	:	Why publish sequences?
	Andreas Pluckthun	:	What are the coming alternatives ?

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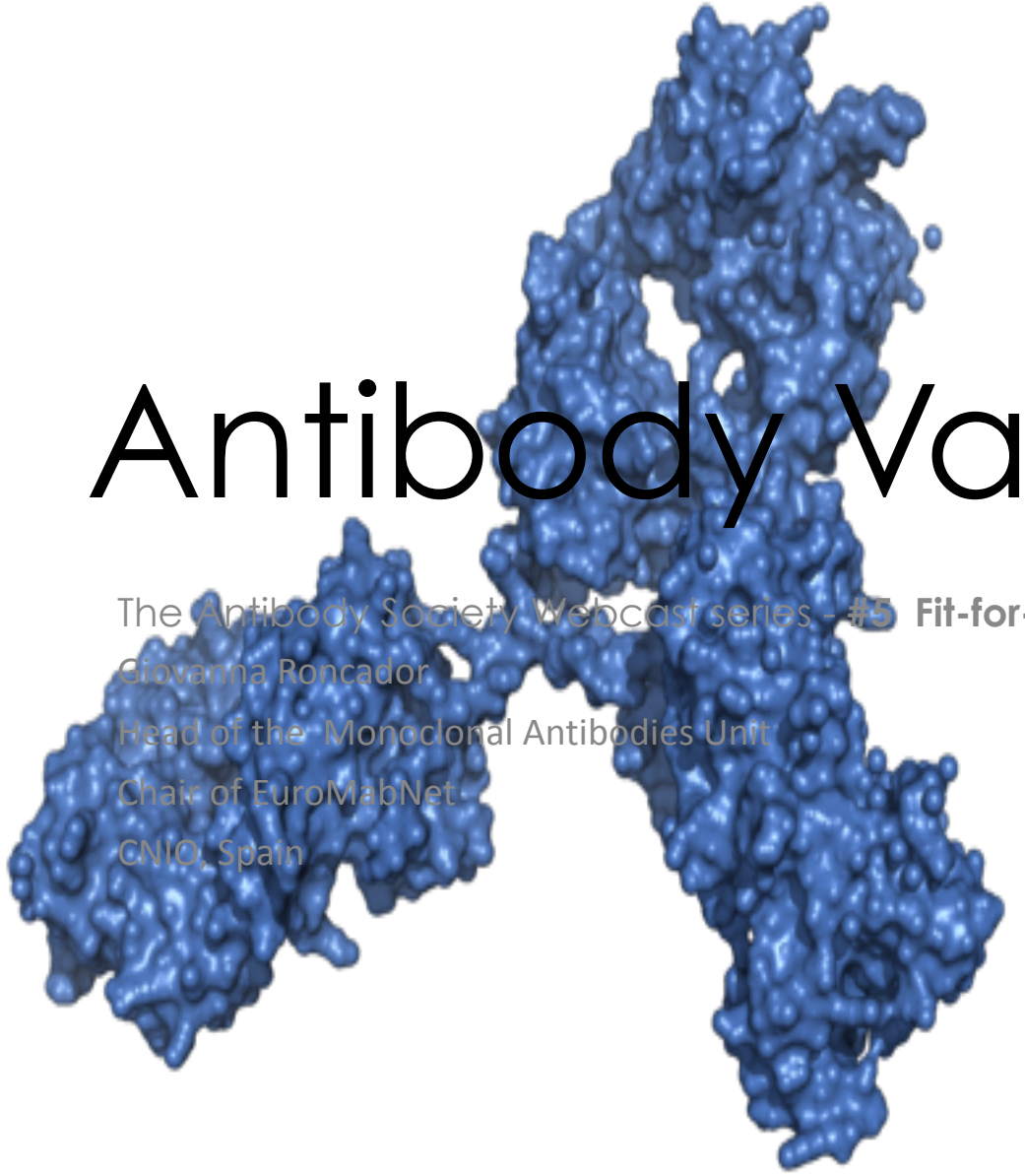

Antibody Validation

The Antibody Society Webcast series - #5 **Fit-for-Purpose Positive and Negative controls**

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Chair of EuroMabNet
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Fit-for-purpose Positive and Negative controls

The Antibody Society Webcast series – Antibody Validation #5

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Head of the Monoclonal Antibodies Unit

Chair of EuroMabNet

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What is the goal of antibody validation?

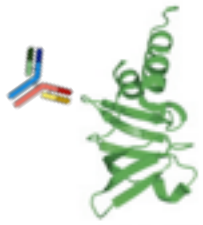
Is to demonstrate that the Ab is **specific**, **selective**, and **reproducible** **in the application in which it is going to be used.**

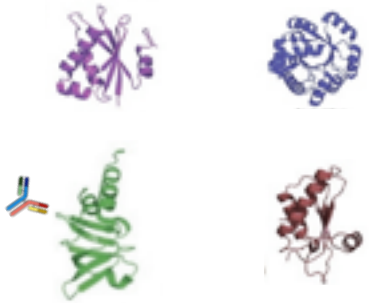
Specificity + **Selectivity** + **Reproducibility**

capability to bind specifically to one **unique epitope**

ability of an Ab to react only with **one antigen**

ability to **duplicate results** over long periods of time by different laboratories.





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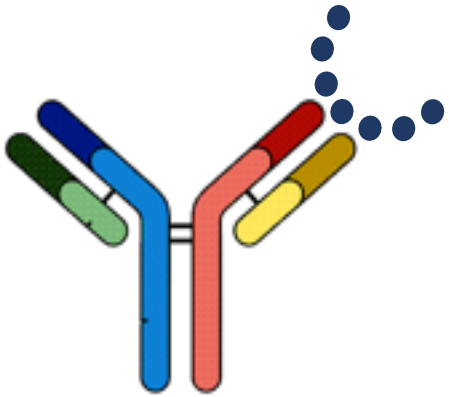
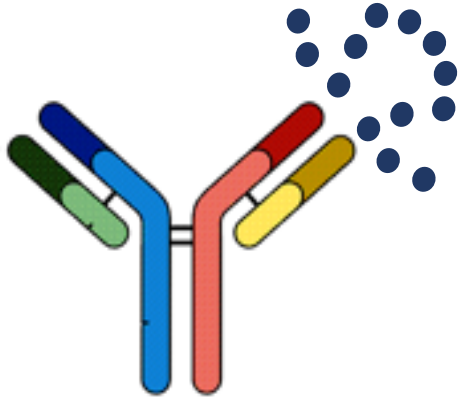
Ab performances is application-specific

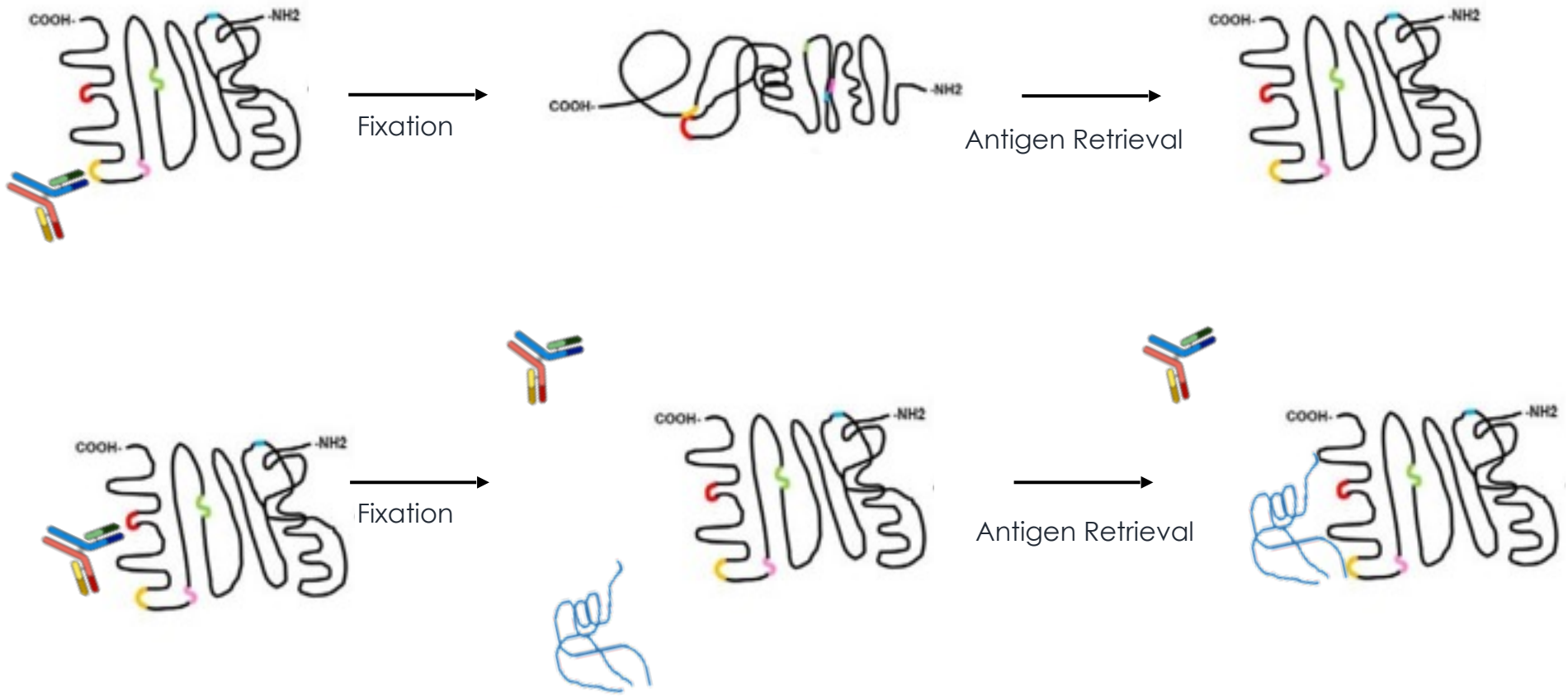


Linear Epitopes Western Blotting, IF, IHC



Conformational Epitopes ELISA , Flow Cytometry, IP

Conformational and linear epitopes of native/partially-unfolded proteins (fixed & cross-linked)



Picture from Ramos-Vara and Miller Veterinary Pathology 2014

Before starting Ab Validation: review your target

Full literature review of your target

- Build a picture of when and where expression is expected (expected sub cellular localization)
- Discover possible cross reactivity with related proteins
- Identify all known variants
  - alternative splicing,
  - proteolytic cleavage
  - post-translational modification

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Importance of +/- controls

The **key** to proving antibody specificity is often the correct use of controls

Positive control:
should confirm that your target antigen is expressed on cells and tissues

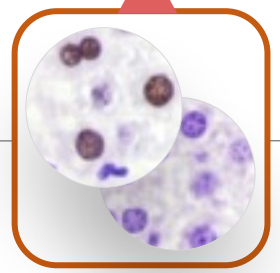
Negative control:
should consist of tissues or cells where your target protein is known to be absent

Positive and Negative controls

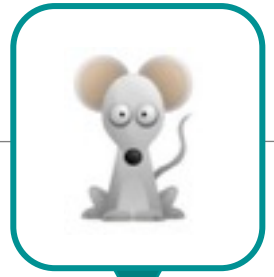
Cell lines and tissues

Transfected cells

Cells with different levels of protein expression

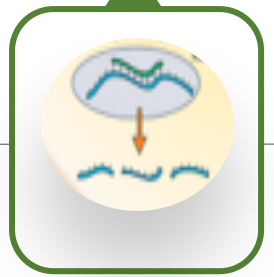


Knockout mice

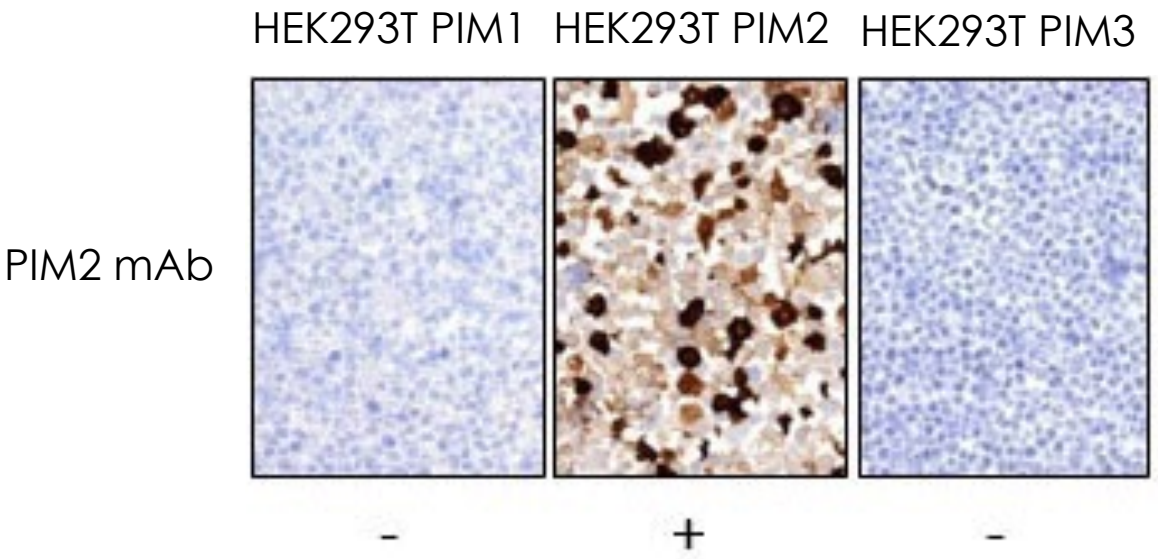


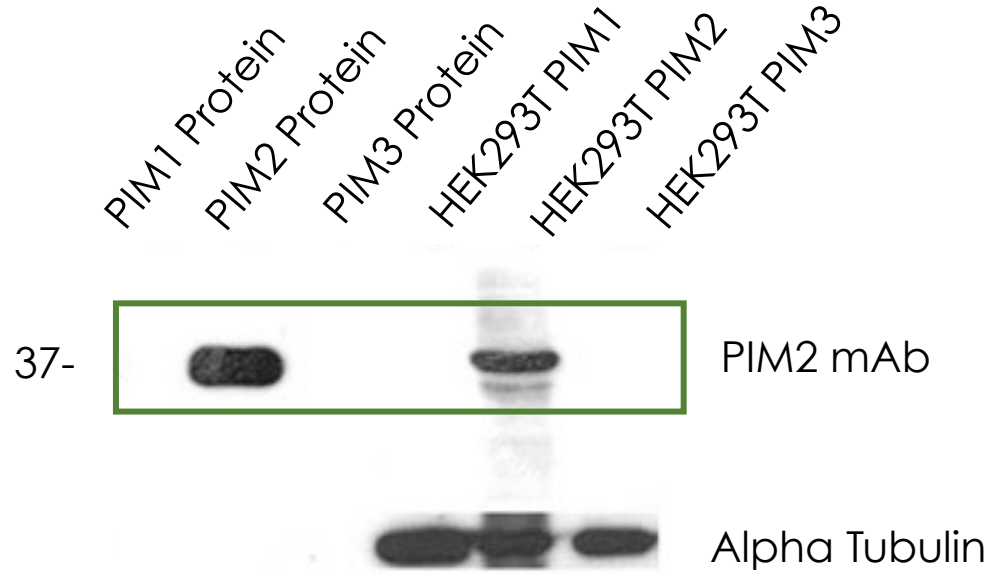
siRNA

CrisperCas9



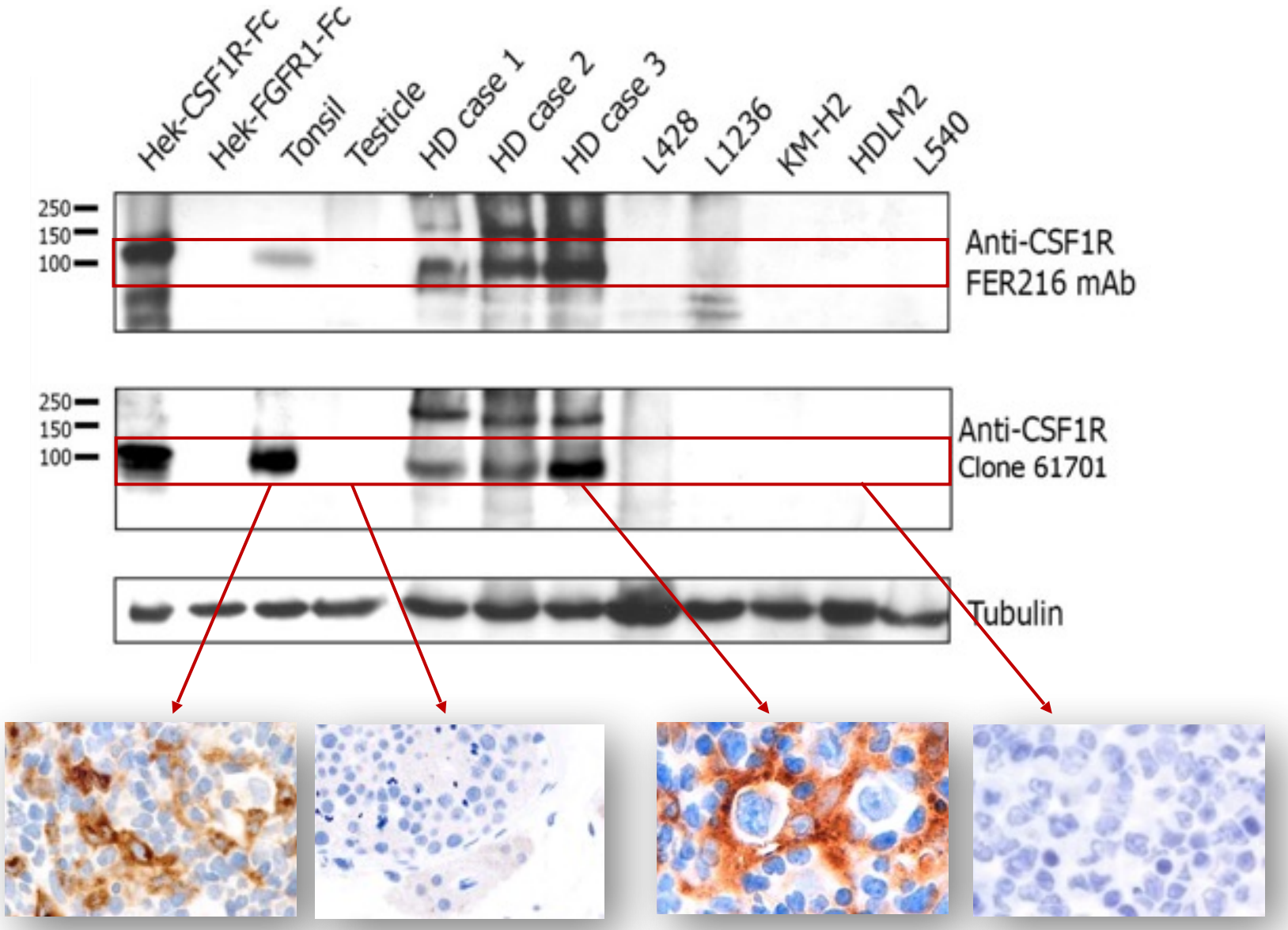
Transfected cells are a useful tool to check Ab cross reactivity



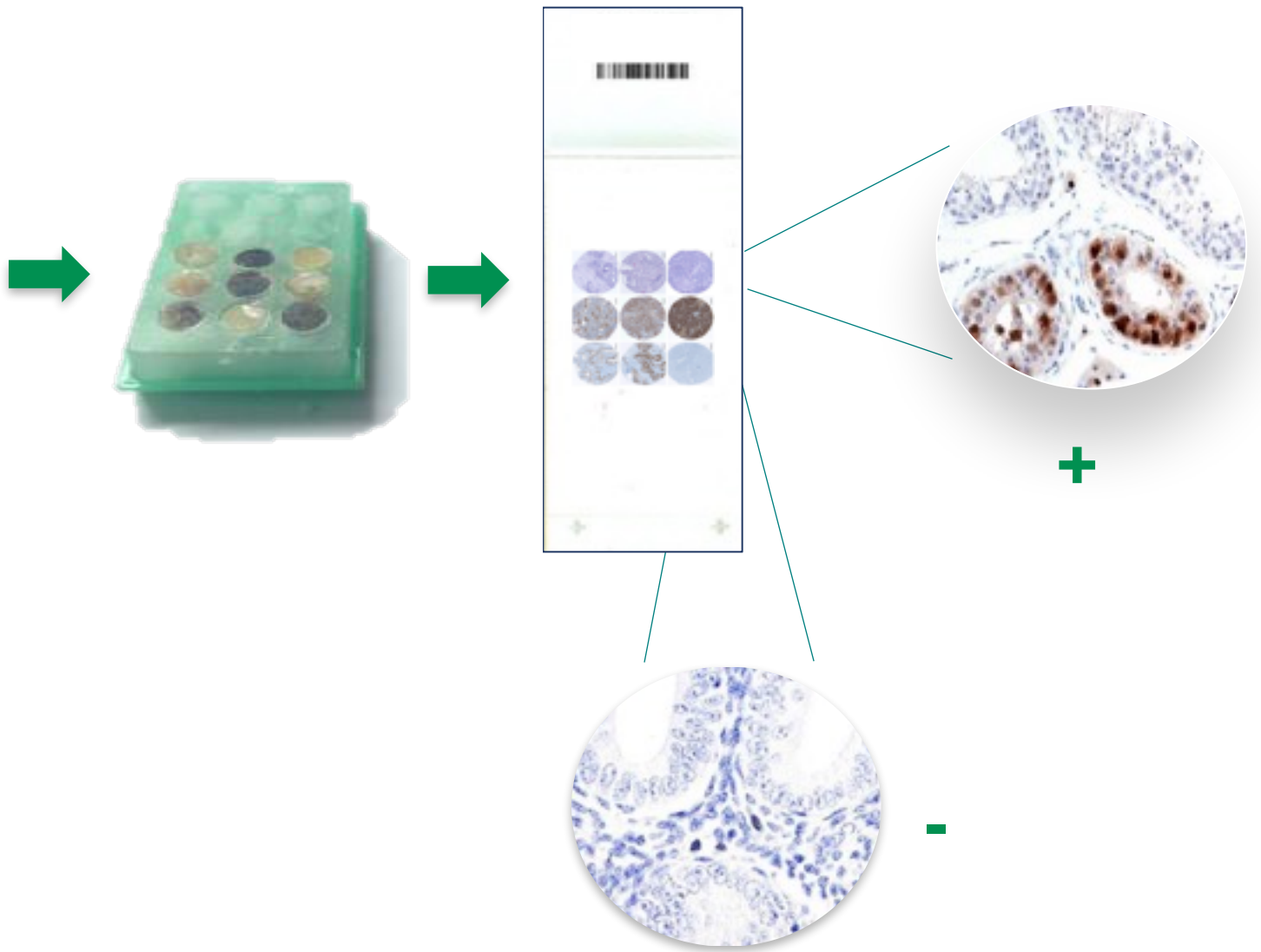


In some cases Abs that recognise it target in transfected cells may still be unable to detect the native endogenously expressed protein

+/- controls: example Western Blotting

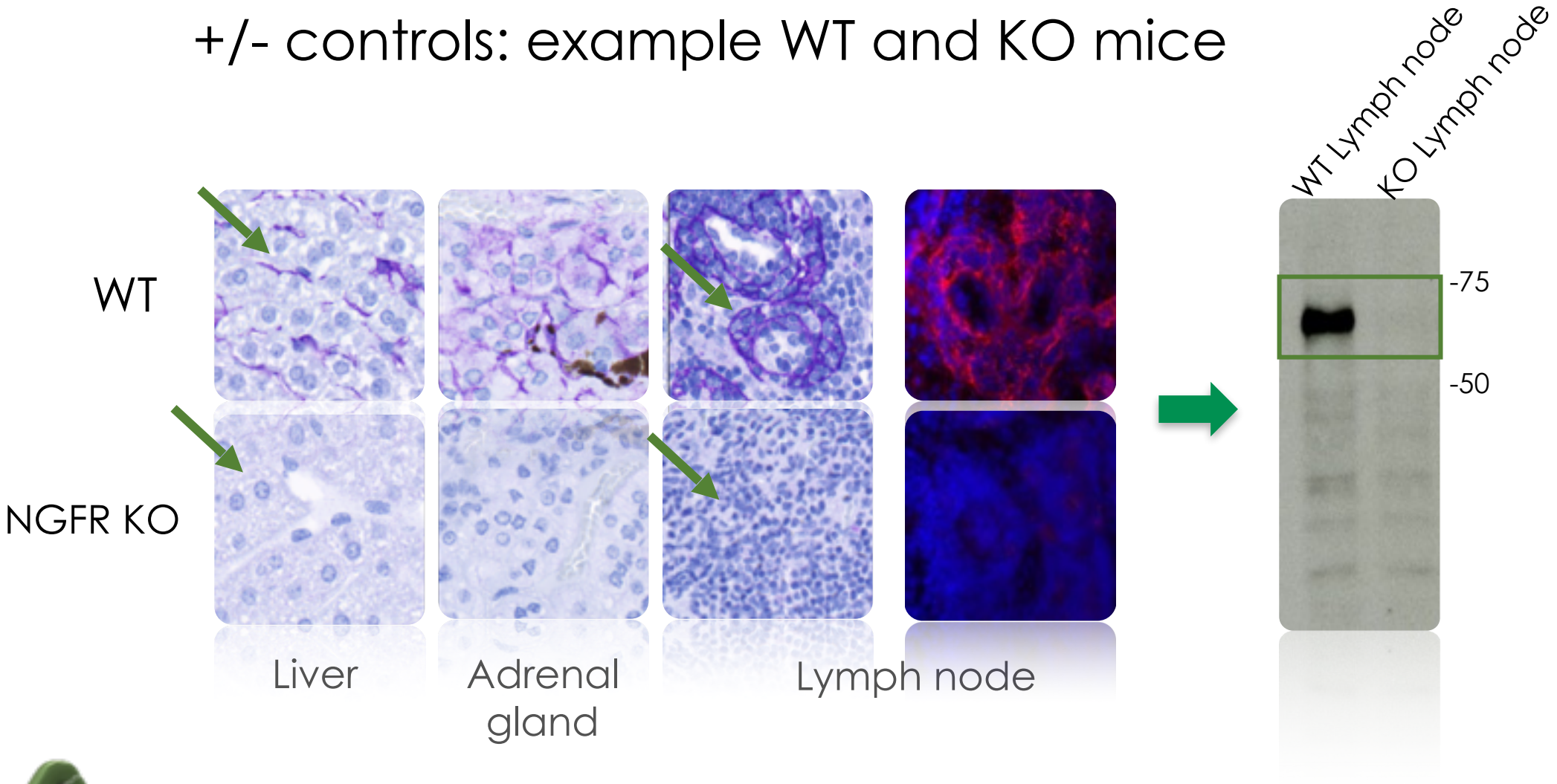


Small tissue arrays are a useful tool for Ab validation by IHC



- Human tissue blocks
- Cell lines blocks
- Transfected cells blocks
- Tumour blocks

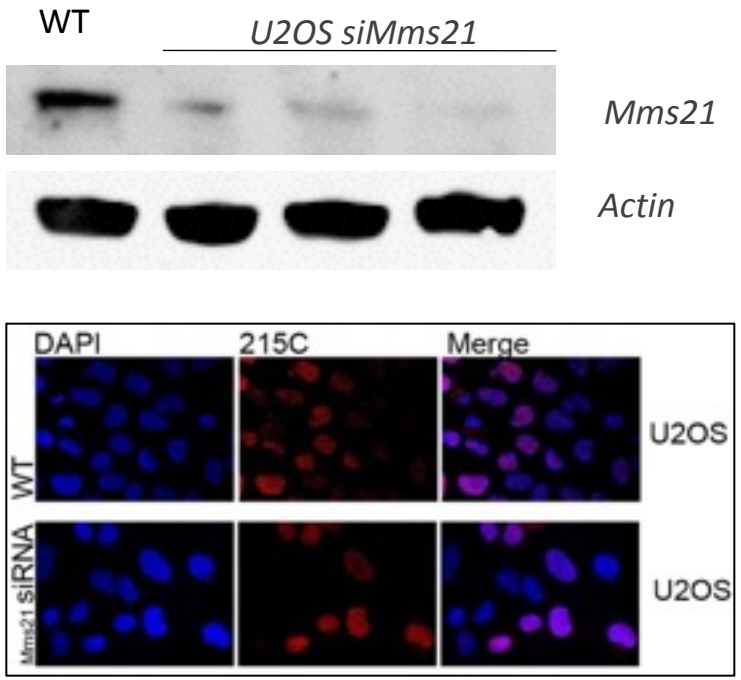
+/- controls: example WT and KO mice



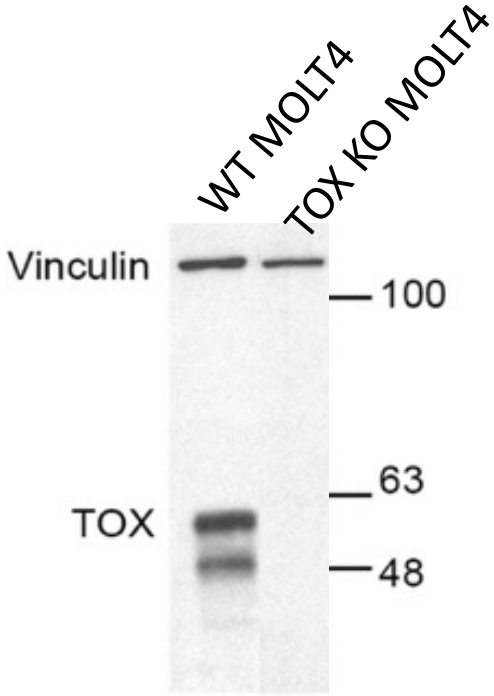
The use of KO mice is only applicable if your antibody recognizes a mouse molecule.

+/- controls: knock down and knock out methods

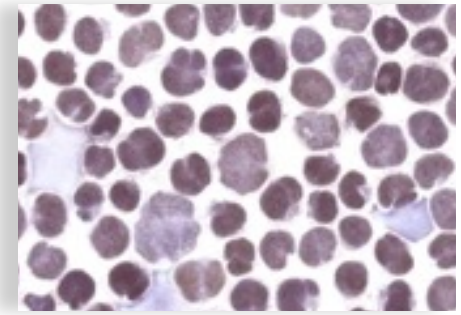
Example siRNA:
Ab against Mms21



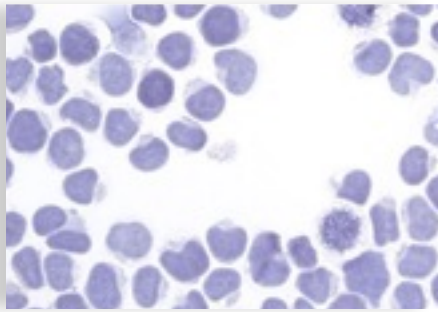
Example CrisperCas9:
Ab against TOX



WT MOLT4



TOX KO MOLT4



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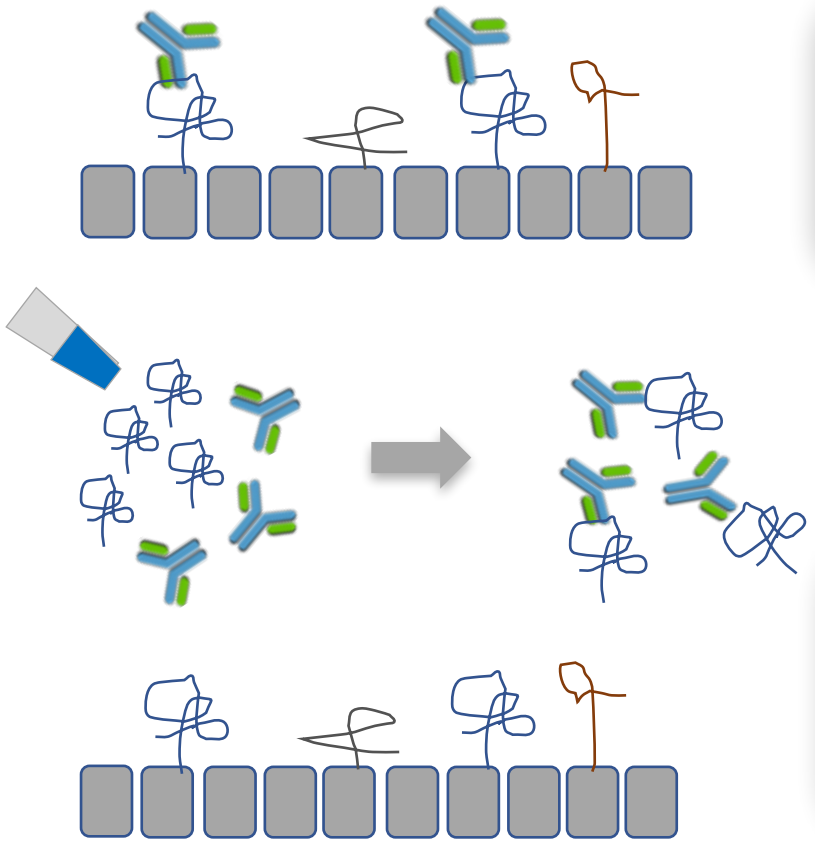
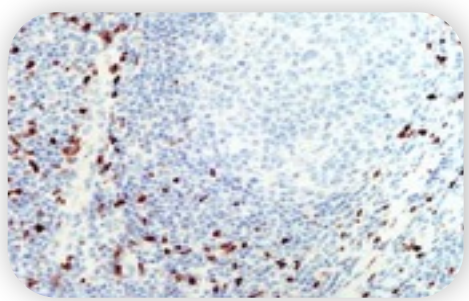
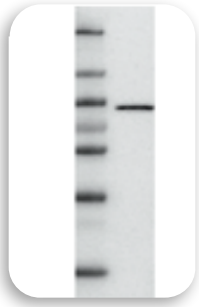
Antigen Preadsorption do not determine specificity



MNDA Ab

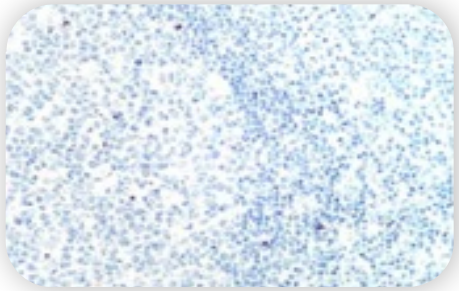


IHC tonsil



WB tonsil extract

MNDA + Antigen absorption



IHC tonsil

WB tonsil extract

Blocking reactivity with excess antigen demonstrates that the reactivity is produced by an antibody that recognizes that antigen and **DO NOT** prove specificity.

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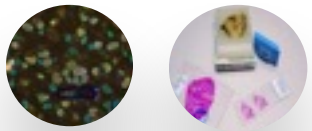
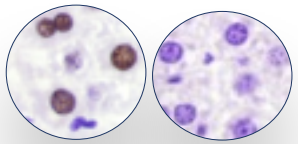
Summary

Cell lines and tissues

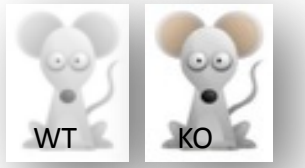


Transfected cells

different levels
of protein expression



Knockout (KO) mice



Haploid KO cell models

Use more that one
Abs
against your target

- IHC
- WB
- IP
- Flow Cytometry
- CHIP
- Other Techniques

Use more than one technique

Confirm that the result obtained fit with how described in the literature

The validation data should be available to the scientific community.

Publications

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Presented by Giovanna Roncador

Produced and Directed by Simon L. Goodman

Production Manager Fran Breden

Writen by Simon Goodman

https://www.antibodysociety.org/

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