

It's a Knockout

The Antibody Society Webcast series – Antibody Validation #7

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abcam

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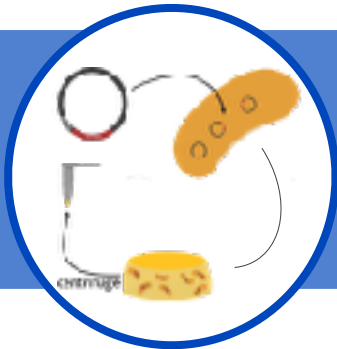
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Antibody development and characterisation have a great impact on antibody specificity, performance and consistency

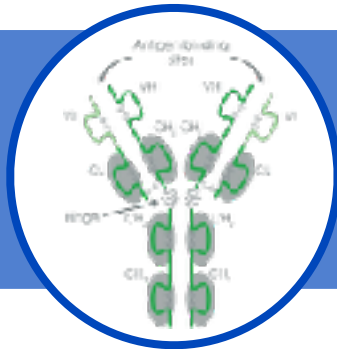
Target
selection &
Protein
Design



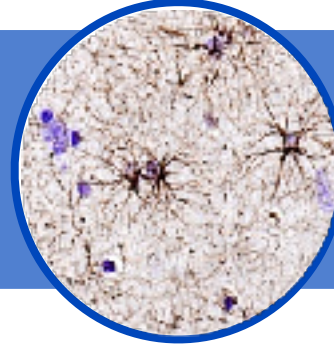
Protein
Expression
and QC



Antibody
Discovery



Antibody
characterisation



Batch to
batch QC



Best-in-class antibody discovery platforms supports generation of highest performance antibodies

Antibody discovery driven by a broad range of platforms & screening approaches



Rapid RabMAb®



B Cell Cloning



NGS

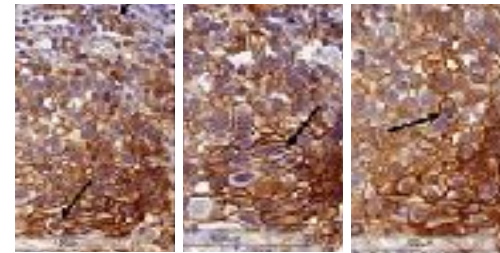
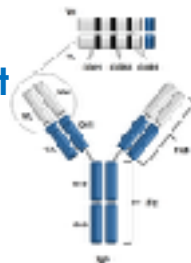


Phage display

... improved sensitivity, specificity and reproducibility

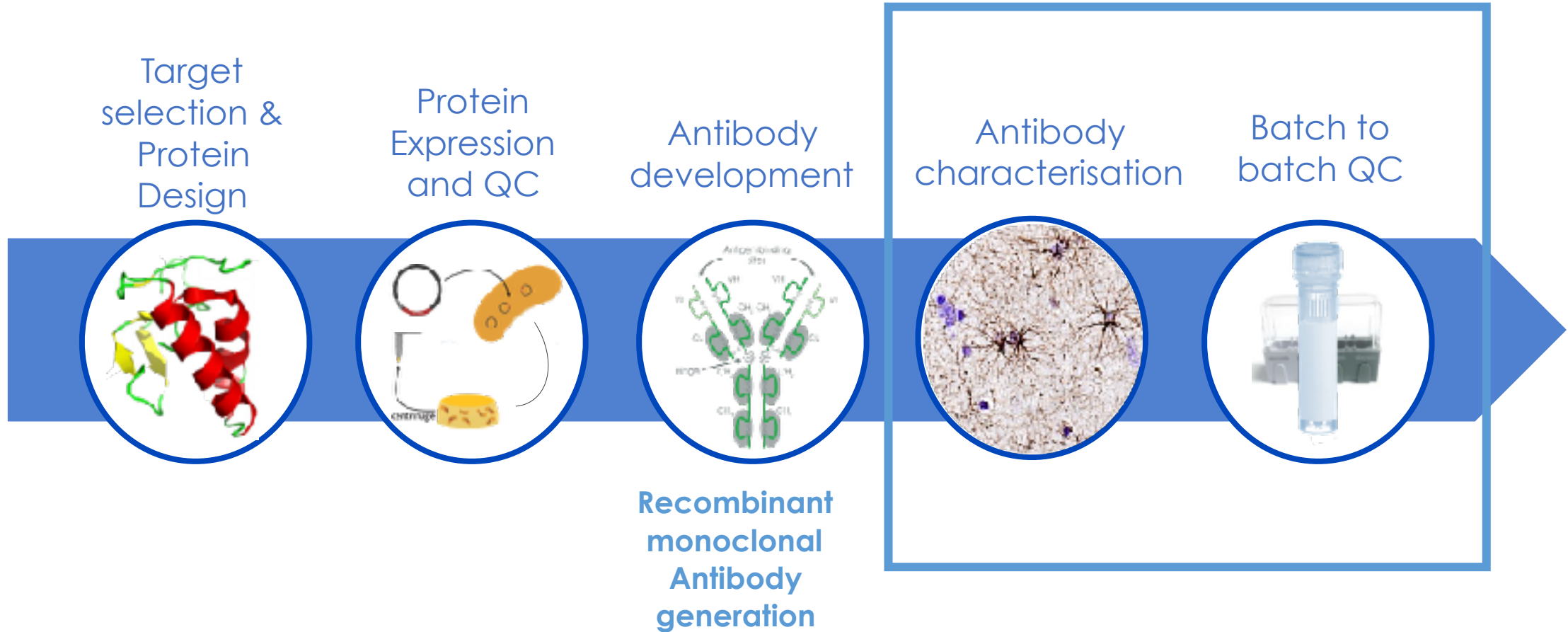
Over 18,000
recombinant
RabMAbs

**Recombinant
rabbit
monoclonal
antibody**



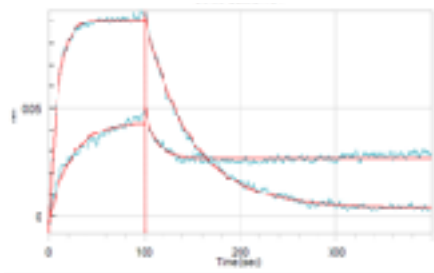
Batch - batch consistency

Antibody development and characterisation have a great impact on antibody specificity, performance and consistency

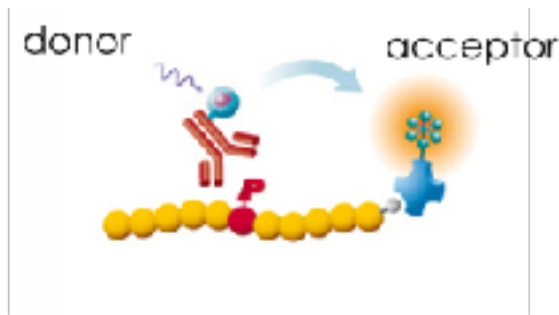


Extensive antibody characterisation and validation is key to antibody specificity and performance

Label free
Off-rate ranking



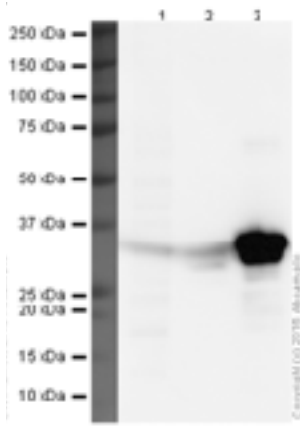
Homogeneous HT assay
development and
screening



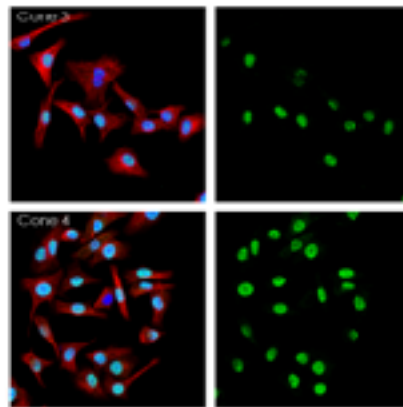
ELISA



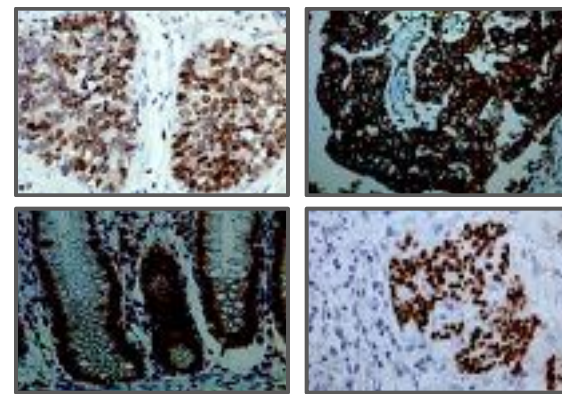
WB



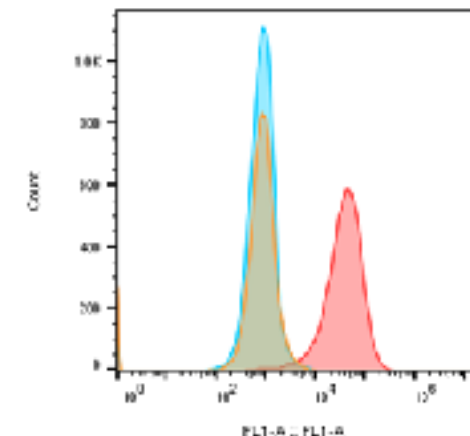
ICC



IHC

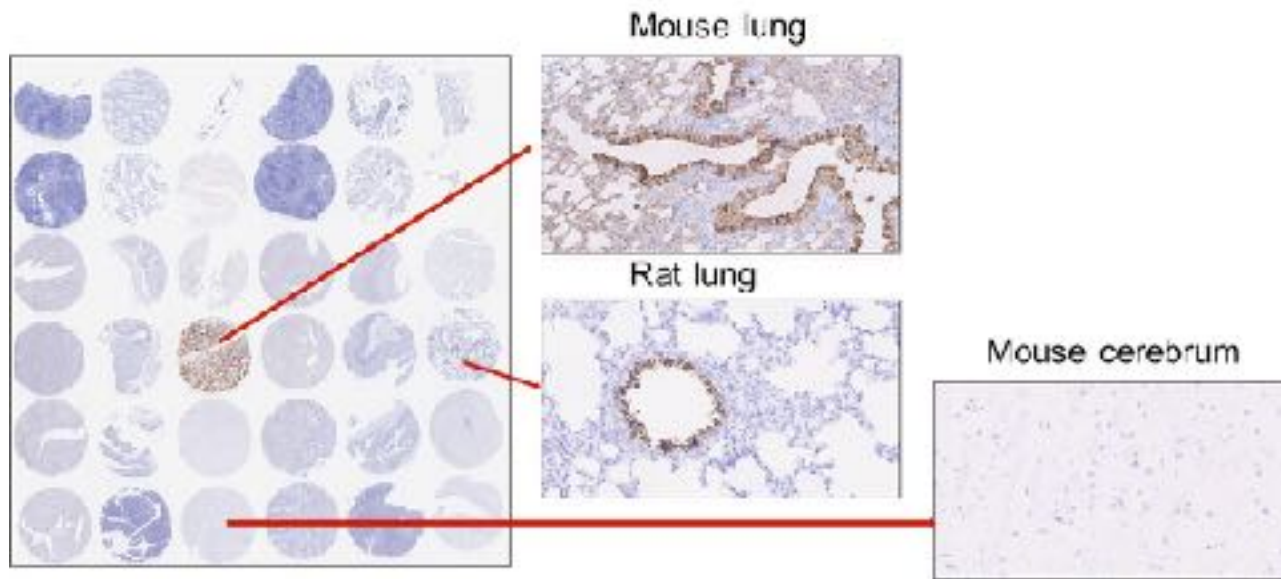


Flow cytometry



Tailored approach to verify antibody specificity

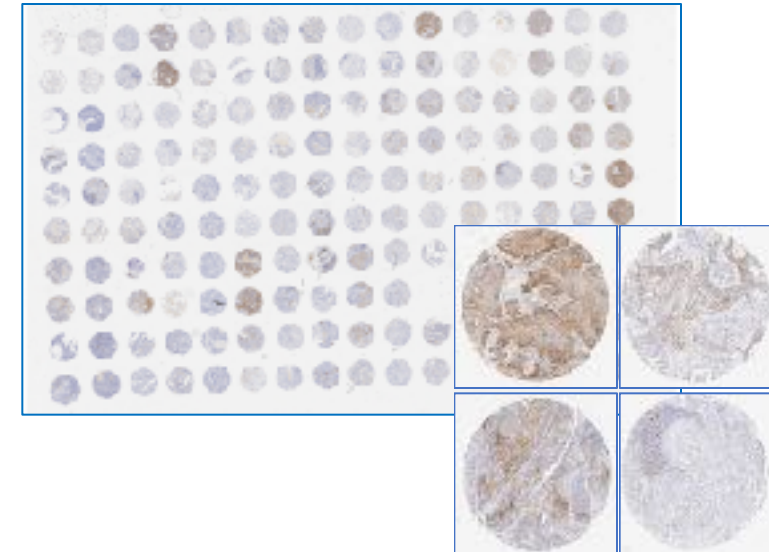
IHC validation on multispecies and multi-tissue microarray



Recombinant RabMAb

Recombinant Anti-Uteroglobulin antibody [EPR19846] (ab213203)

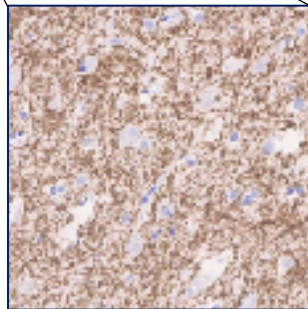
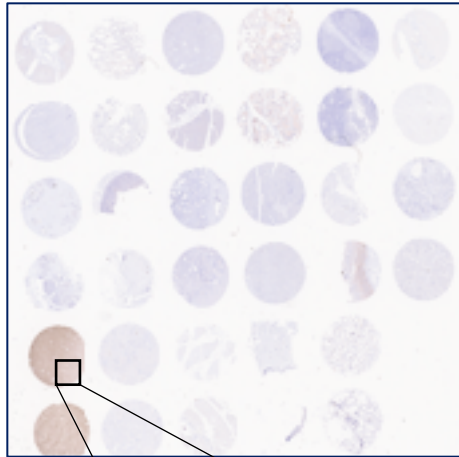
Enhanced validation level - extended to relevant tumour tissue NSCLC TMA



Recombinant anti- PD-L1 (CAL10 ab237726)

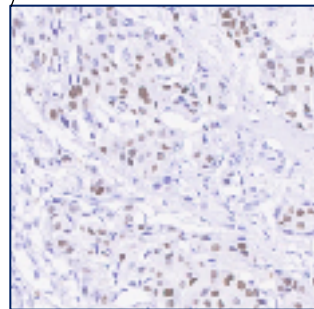
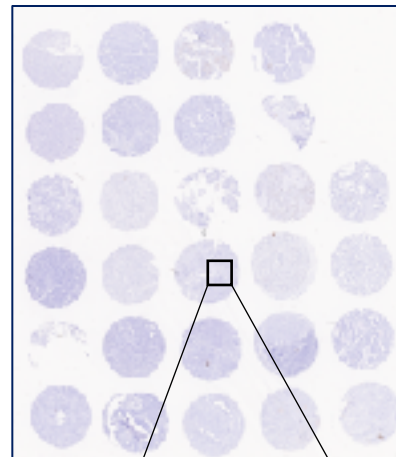
Positive/negative control tissue demonstrates sensitivity and confirms specificity

Multi-human normal TMA



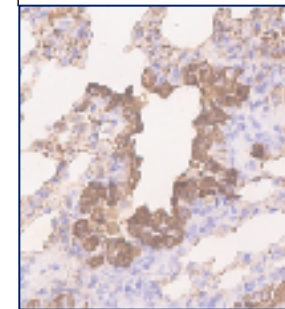
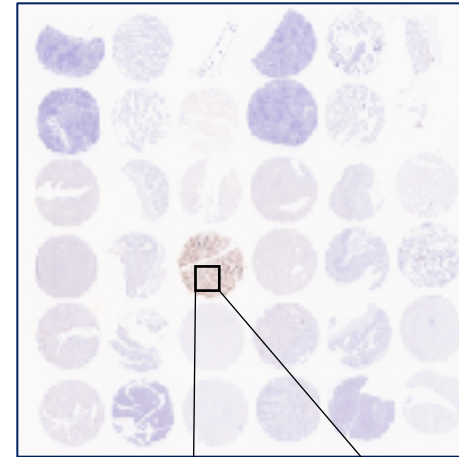
Myelin Basic Protein
(ab218011)

Multi-human tumour TMA



Progesterone Receptor
(ab16661)

Mouse/Rat normal TMA



Uteroglobin
(ab213203)

Evolving & adopting more robust validation technologies

Knockout models represent true negative controls

Wild type



KO

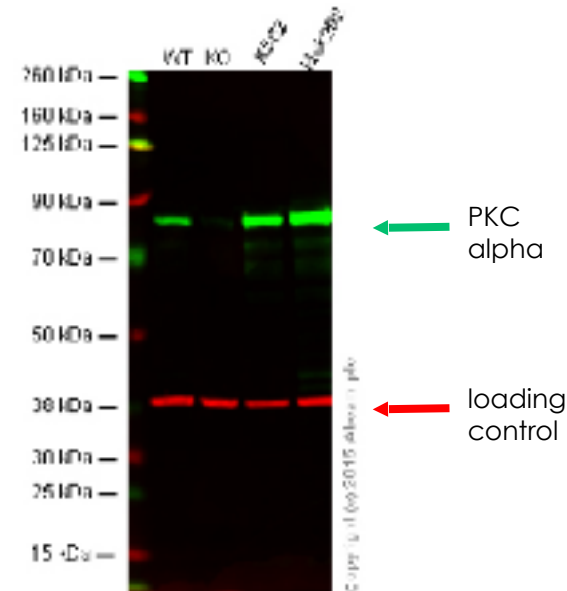


Additional control cells



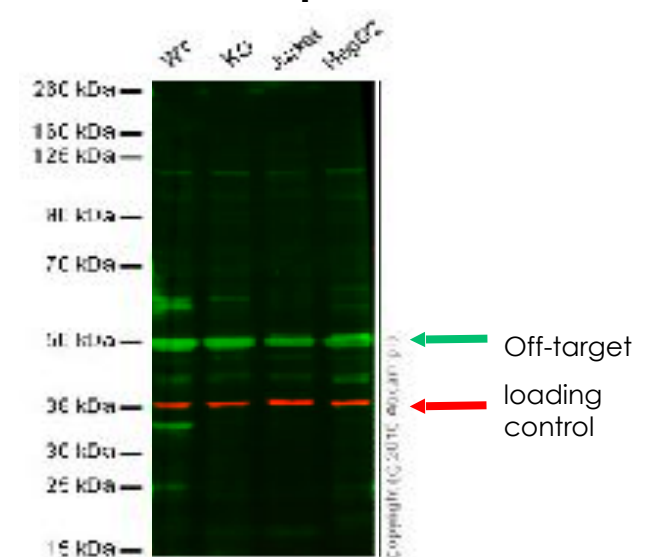
Selection of
several
antibodies to
test

**Specific for target of
interest**



Anti-PKC alpha recombinant
RabMAb® antibody [Y124] ab32376

**off-target – a signal in the
KO sample**



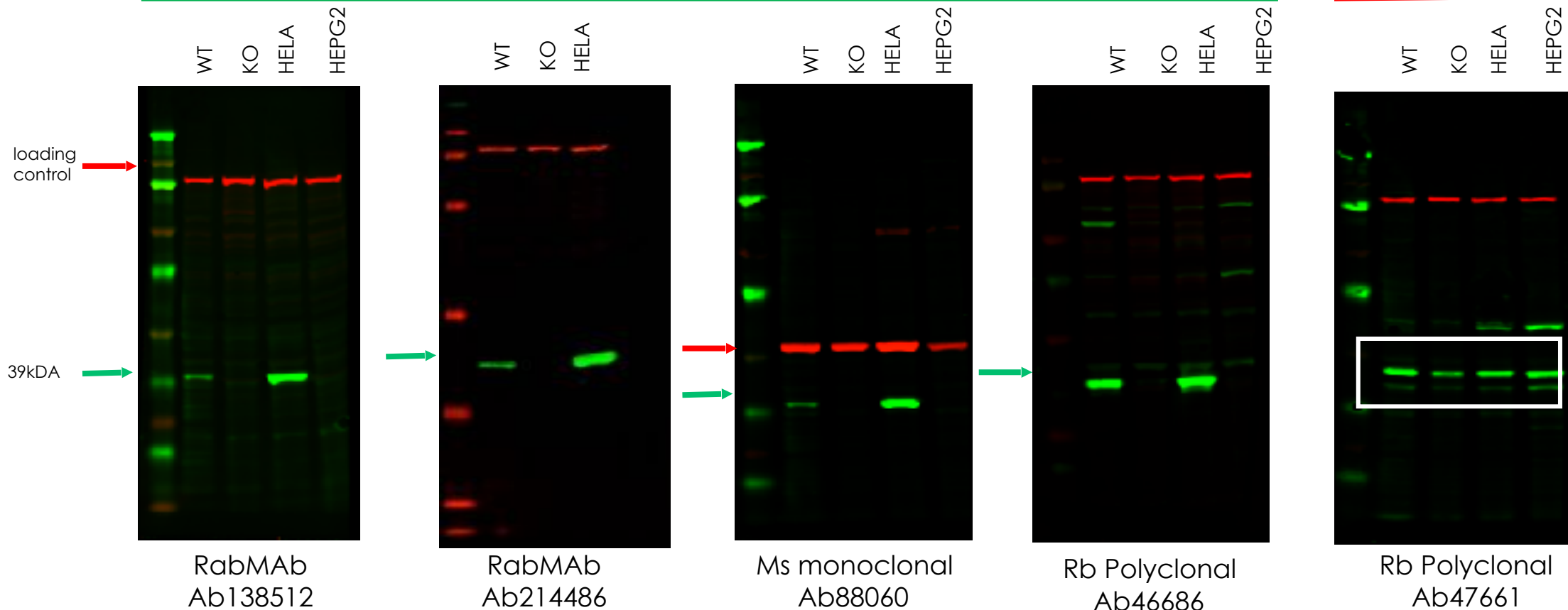
*Anti-Integrin linked ILK
Rabbit PAb ab106955

Extensive validation using multiple antibodies per target

Annexin A1/ANXA1

KO VALIDATED

Product no longer available



Anti-Annexin A1/ANXA1 antibody (ab47661) is not available

Specificity confirmed by KO validation for **>2500** antibodies

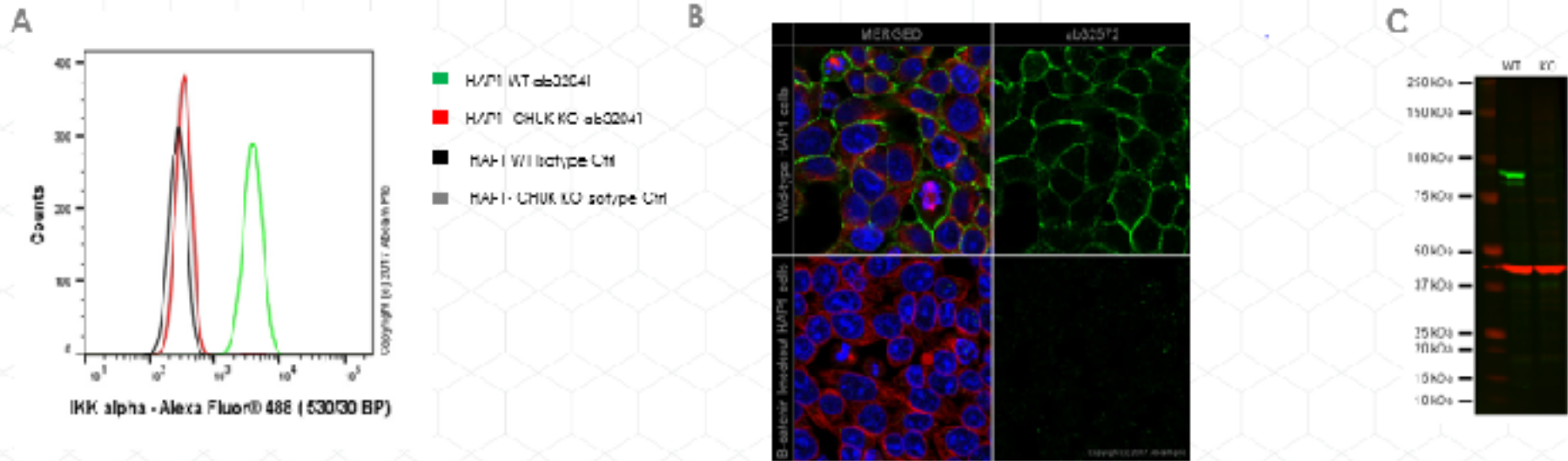


Fig.1 Knock out testing of anti IKK alpha antibody (ab32041). ab32041 was shown to specifically react with IKK alpha when IKK alpha knockout samples were used by Flow cytometry (A), Immunocytochemistry (ICC) (B) and Western Blotting (WB) (~ 04kD) (C).

...large scale KO validation in multiple applications

90

KO flow cytometry
tests

249

KO ICC
tests

7064

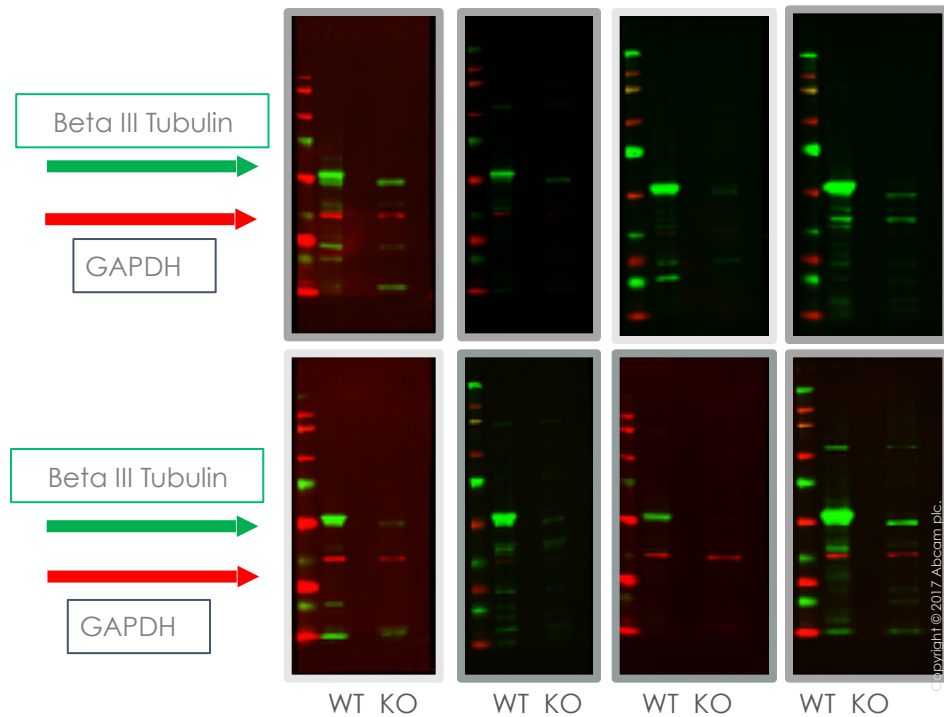
KO WB
tests

2011

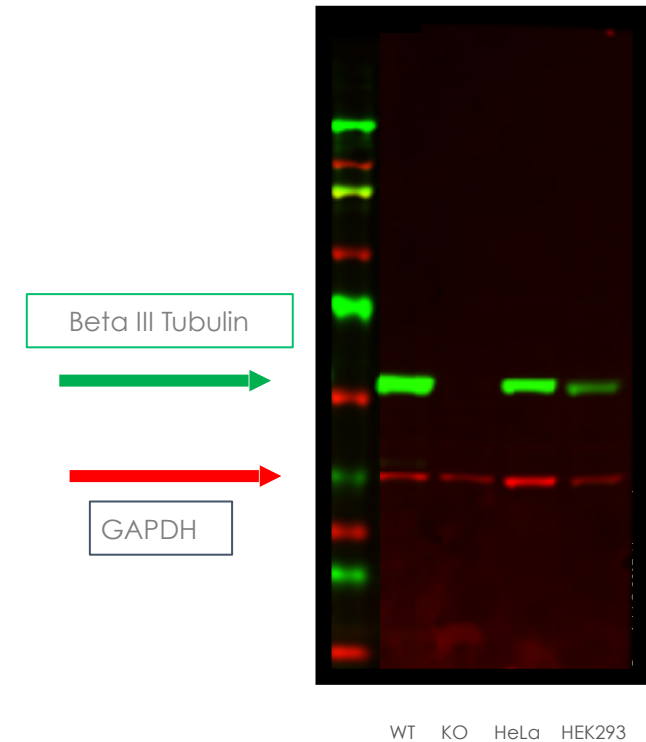
Protein targets
covered

Impact of antibody format on specificity and consistency

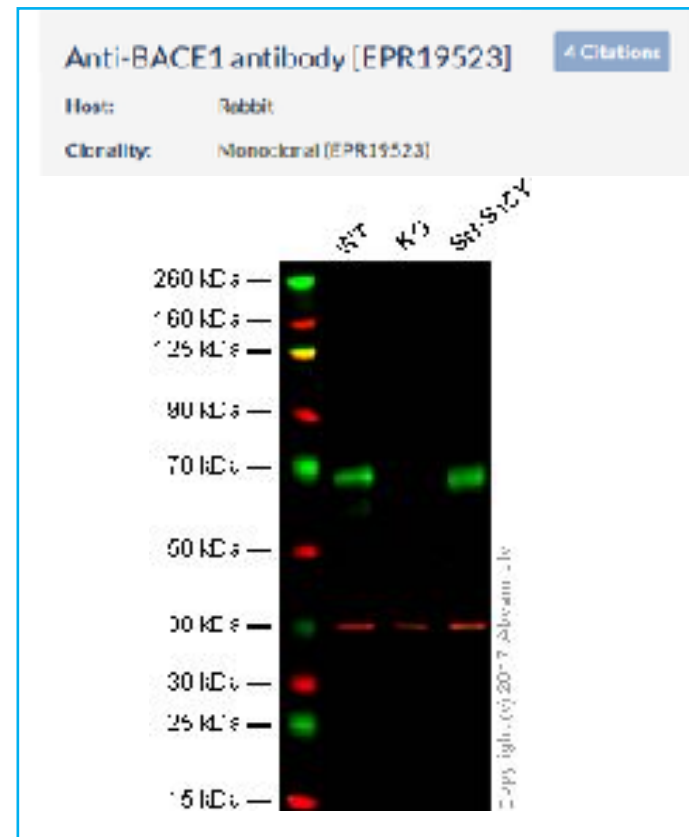
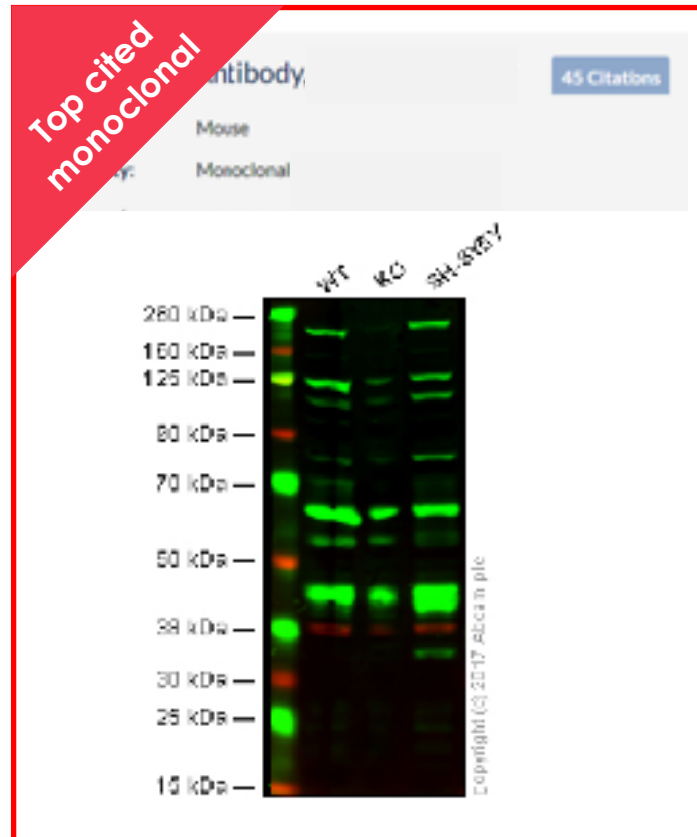
Rabbit polyclonal Anti-beta III Tubulin antibody (ab18207)



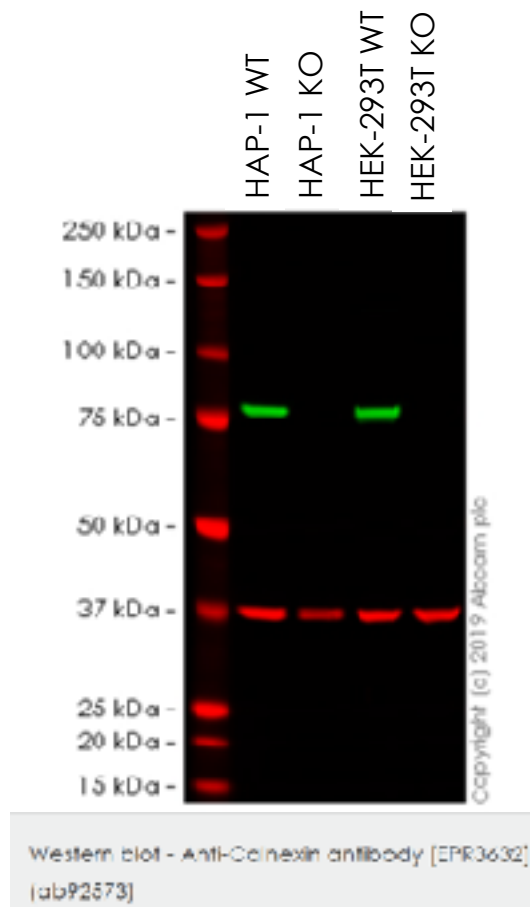
Recombinant Anti-beta III Tubulin antibody [EPR19591] (ab215037)



Top cited clones do not always perform the best

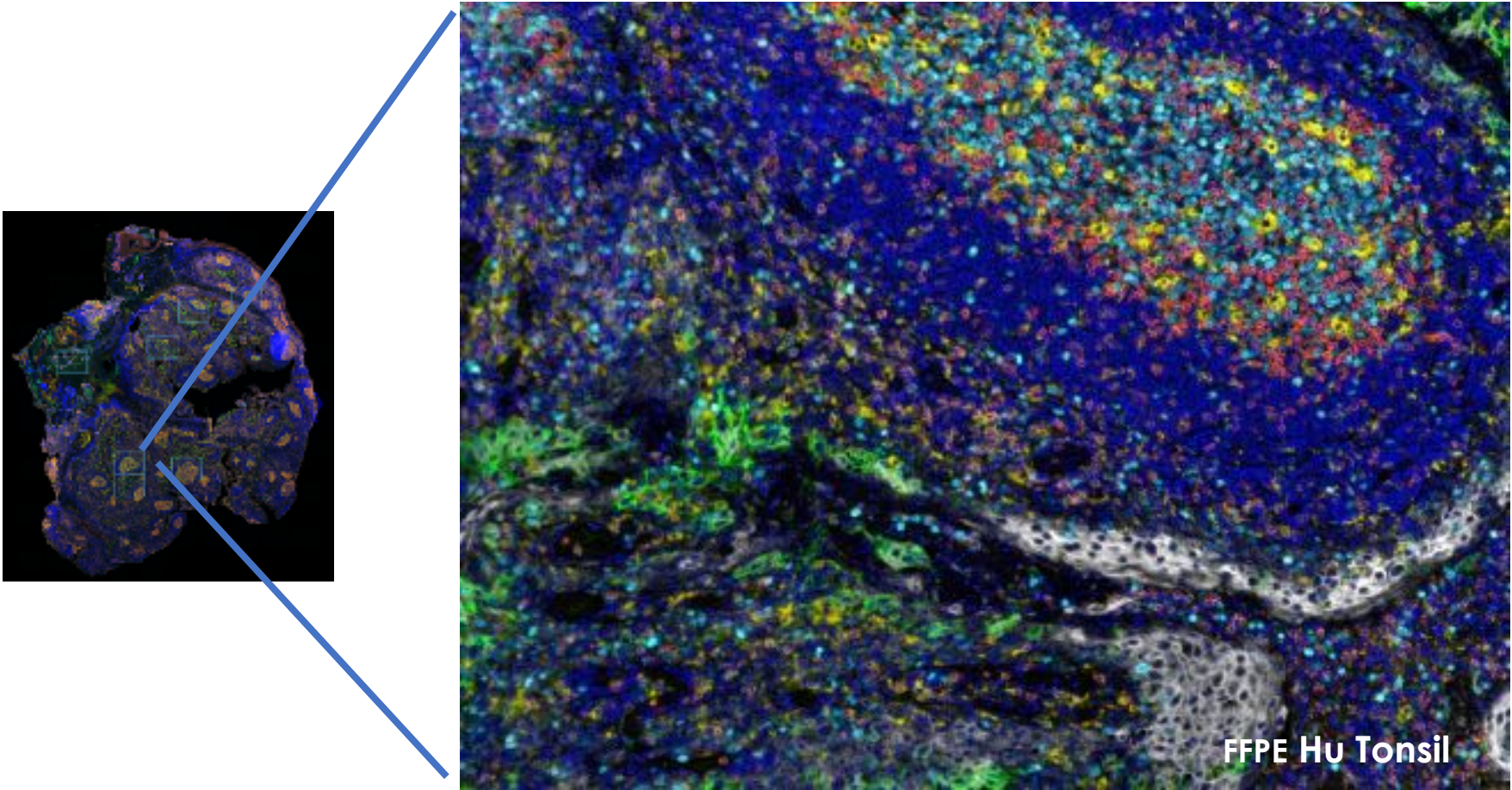


KO lysates and cell lines available



- **Choice:** 2,800 pre-validated paired lysates & diploid lines
- **Quality:** Extensive genomic and proteomic validation
- **Speed:** Immediate use, saving 12-16 weeks

Providing industry leading antibody validation data



| | |
|------------------|------------|
| PD1 (CAL20) | Orange |
| PD-L1 (CAL10) | Green |
| CD68 (SP251) | Yellow |
| CD3 (SP7) | Red |
| Ki67 (SP6) | Light blue |
| PanCK (C11) | White |
| DAPI | Blue |

It's a Knockout – Validation Technologies in Practice #2

The Antibody Society Webcast series – Antibody Validation #7

Alejandra Solache

Abcam, UK.

abcam

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Next Webcast in 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 ErbB 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 ?

Validation of Commercial tool Antibodies

It's a Knockout – Validation Technologies in Practice #2

The Antibody Society Webcast series – Antibody Validation #7

Presented by Travis Hardcastle and Alejandra Solache

Produced and Directed by Simon L. Goodman

Production Manager Fran Breden

Written by Simon Goodman

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Validation of Commercial Tool Antibodies

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

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It's a Knockout – Validation Technologies in Practice #2

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