



Recombinant monoclonal antibodies: An indispensable research tool

Alejandra Solache

VP New Product Development

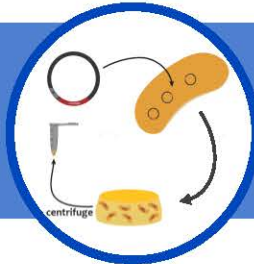
Abcam

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

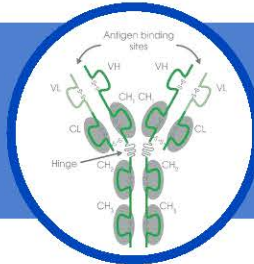
Target selection & Protein Design



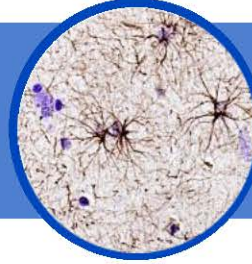
Protein Expression and QC



Antibody Discovery



Antibody characterisation



Batch to batch QC



Advantages and constraints of polyclonal antibodies

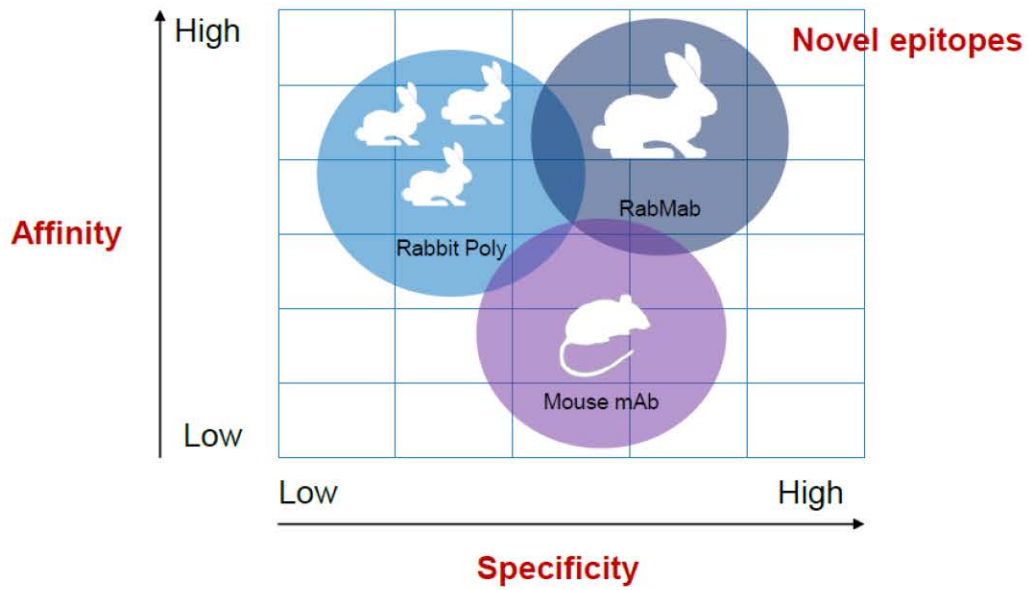
Advantages

- Relatively fast to produce
- Recognize multiple epitopes in a single target
- Useful for certain applications, IP

Constrains

- Recognize multiple epitopes in a single target
- Cross-reactivity with homologous proteins
- Low sensitivity and specificity
- Batch-to-batch variability
- Initial discovery and production is *in vivo*

Compared to other common antibody platforms, rabbit monoclonal antibodies have the highest affinity and specificity



Advantages and constraints of hybridoma-derived monoclonal antibodies

Monoclonal (hybridoma) antibody

Advantages

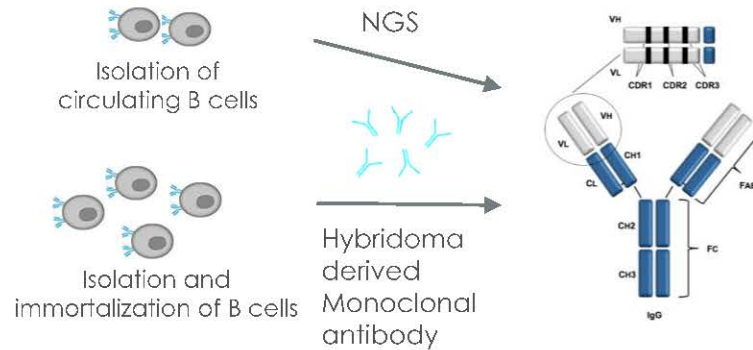
- Recognize a single epitope
- High specificity
- No or low batch-to-batch variability

Constrains

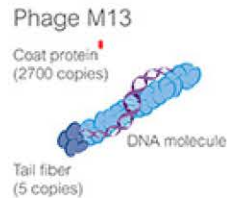
- Vulnerable to stability issues and loss of epitope recognition
- Unable to produce against toxic antigens
- Antibody discovery phase is *in vivo*

Recombinant antibodies produced through a recombinant DNA platform

A) Recombinant monoclonal antibody development - *in vivo* approach



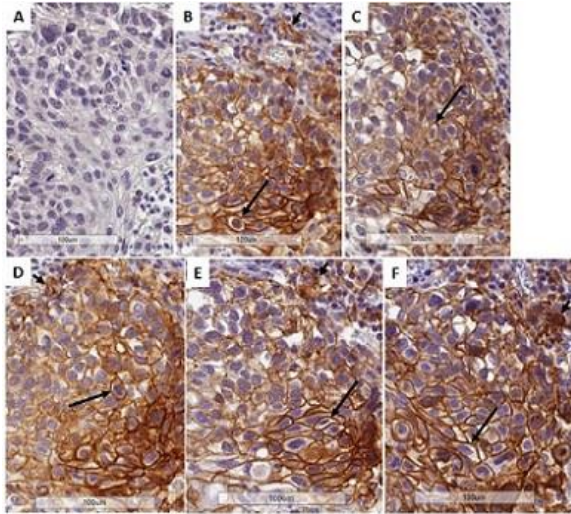
B) Recombinant monoclonal antibody development *in vitro* approach



Axiomx technology
Phage Display
Recombinant antibodies

Recombinant
monoclonal
antibody

Advantages and constraints of immune-based recombinant antibodies



IHC batch testing of human lung NSCLC stained with anti-PD-L1 (ab205921) at 2 µg/mL.

A = Neg control, B -F = different batches. All batches showed consistent results.

Recombinant Monoclonal (immune-derived) antibody

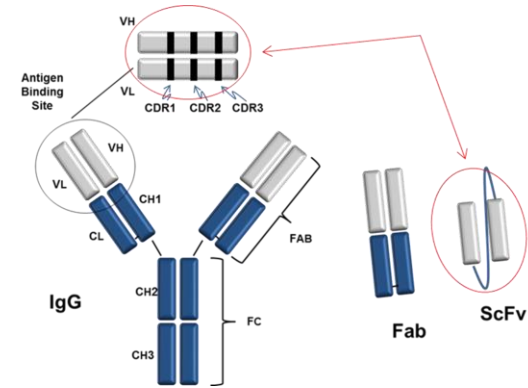
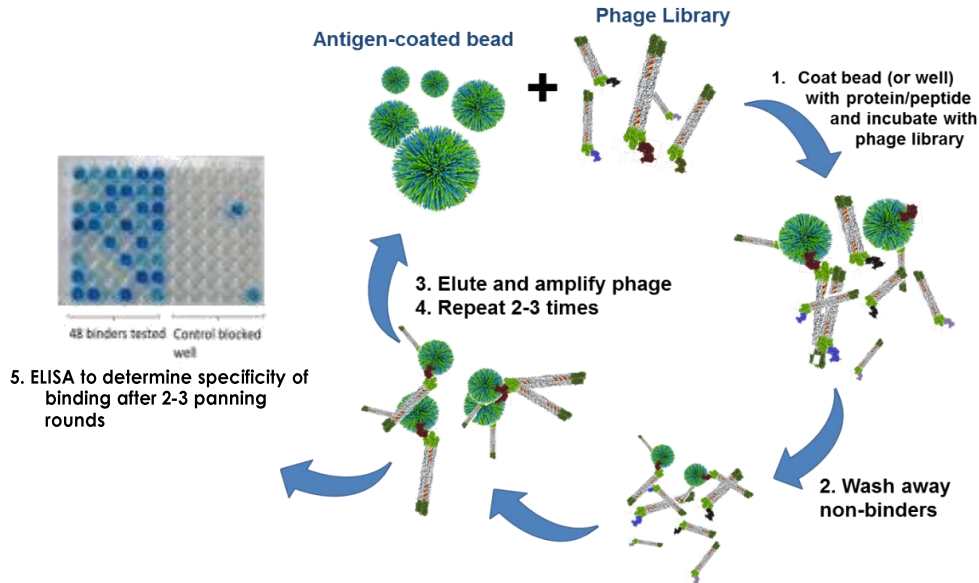
Advantages

- Recognize a single epitope
- Improved consistency and reproducibility
- Improved sensitivity and specificity
- No clone viability issues
- Direct access to sequence
- Fully scalable *in vitro* production

Constrains

- Limited to non-toxic antigens
- Antibody discovery phase not *in vitro*

Recombinant monoclonal antibody development by phage display



Recombinant affinity reagents can be tagged and used as traditional antibodies, or can be recombined back to Fc fusions, Fabs, IgGs, etc.

Advantages and constraints of phage display recombinant antibodies

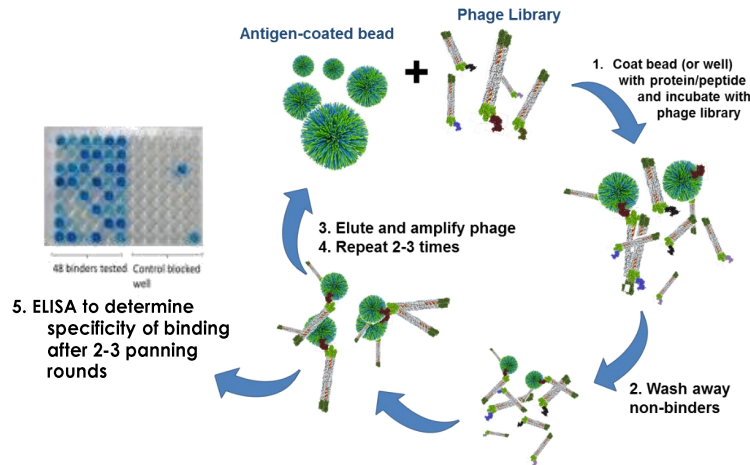
Recombinant (naïve libraries) antibody

Advantages

- Fast process
- Ability to screen human libraries and toxic antigens
- No immunogenicity issue
- No clone viability issues
- Direct access to sequence
- *In vitro* discovery and production

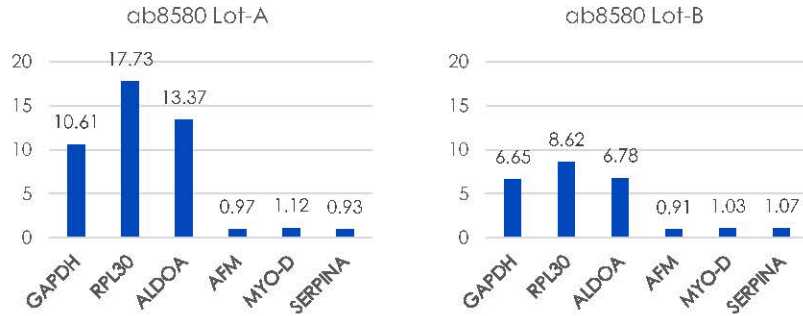
Constrains

- High cost
- Low affinities compared to immunized recombinants
- Difficult to obtain antibodies specific in multiple applications



Performance of Polyclonal vs recombinant monoclonal antibodies to histone modifications by ChIP

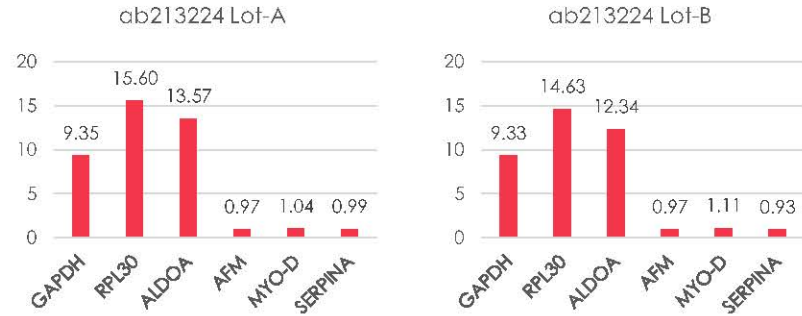
Polyclonal H3K4me3



Variation in signal among batches

RabMAb®

Recombinant monoclonal H3K4me3



There is clear consistency of ChIP data between lots A and B.

Recombinant antibody toolbox

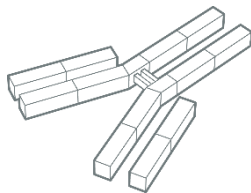
- Three platform options available to create fit for purpose binders



Next Generation Sequencing

NGS antibody selection

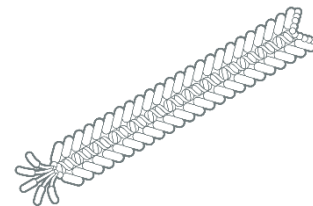
- Full repertoire of antibody sequences to discover rare binders
- Antibody sequence library of >5000 unique IgG sequences



RabMAb[®] discovery

RabMAb rabbit monoclonal antibodies

- High overall affinity
- Broad diversity of epitope recognition

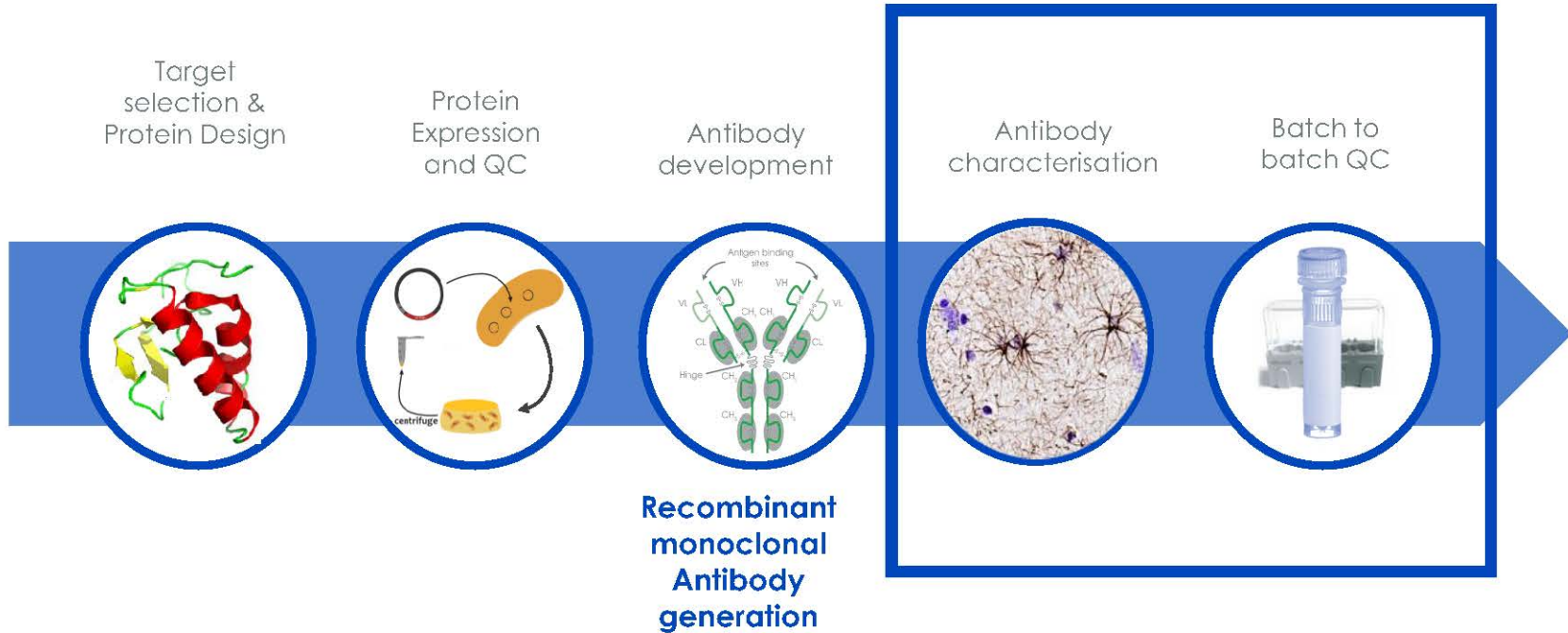


AxiMx[™] phage display

Phage display antibody discovery

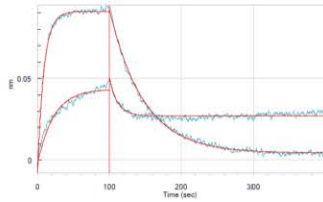
- Animal-free system for generating binders to toxic antigens
- High library diversity to mitigate technical risks

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

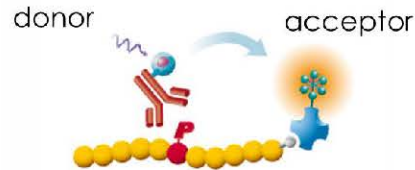


Extensive antibody characterisation and validation is key to antibody specificity and performance

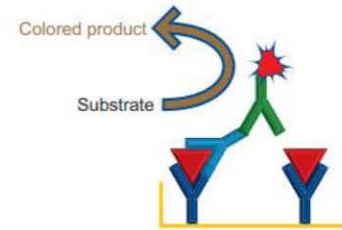
Label free
Off-rate ranking



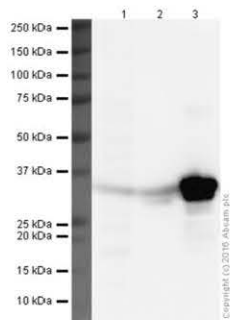
Homogeneous HT assay
development and
screening



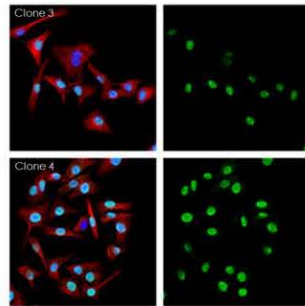
ELISA



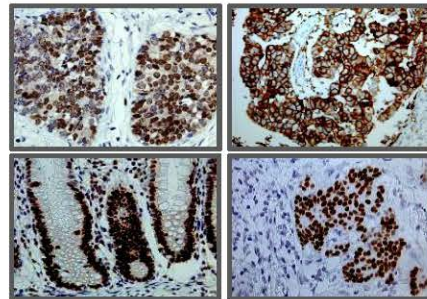
WB



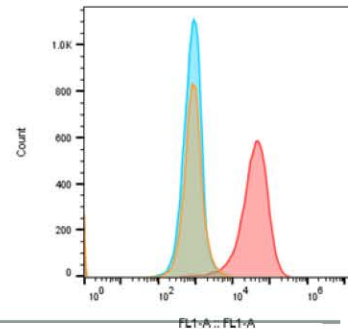
ICC



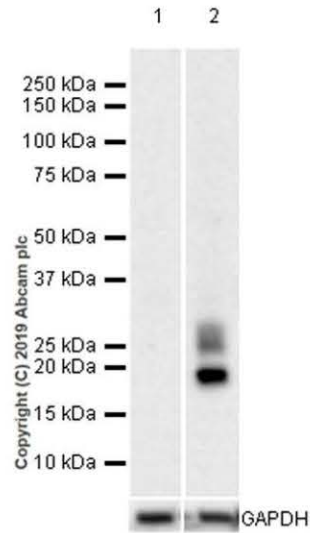
IHC



Flow cytometry

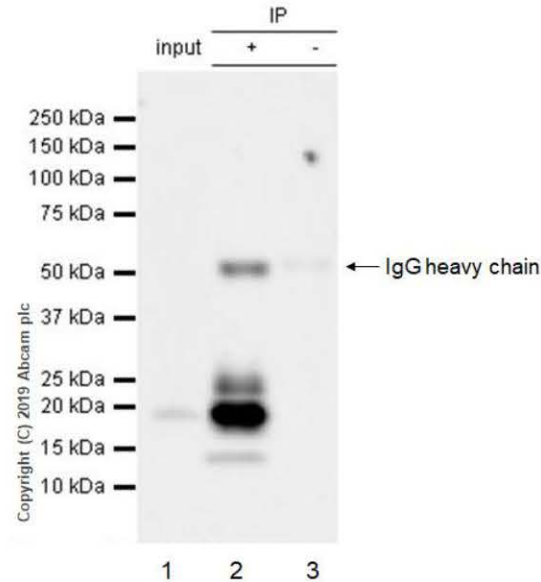


Recombinant anti-BCMA RabMAb[®] perform in multiple applications



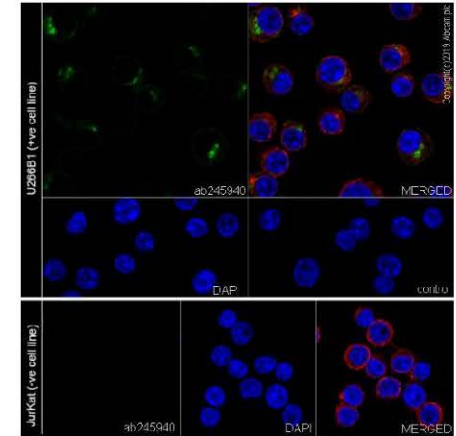
Western blot

MM cell line U266B1

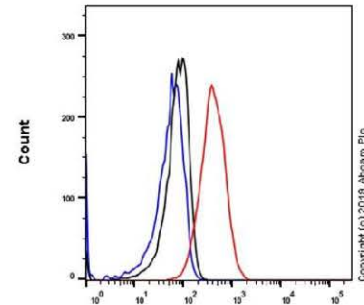


Immunoprecipitation

Ab245940 anti-BCMA



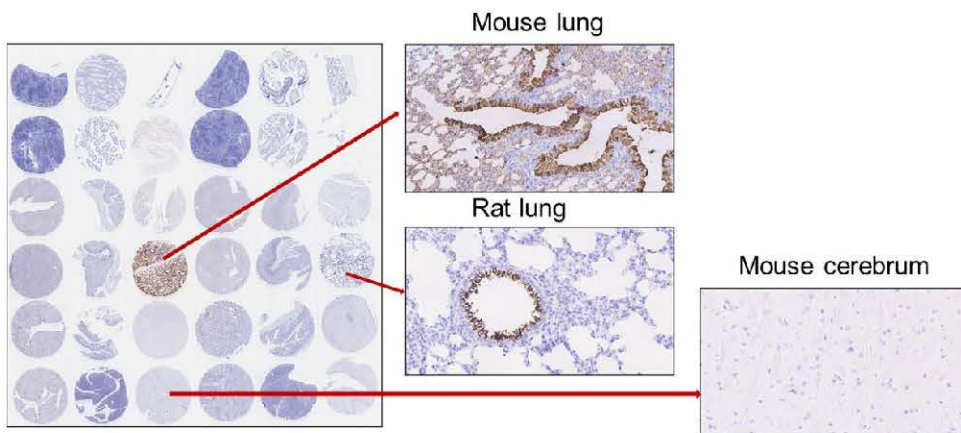
ICC



BCMA - Alexa Fluor[®]488 (530/30BP)
Flow Cytometry

Tailored approach to verify antibody specificity

IHC validation on multispecies and multi-tissue microarray

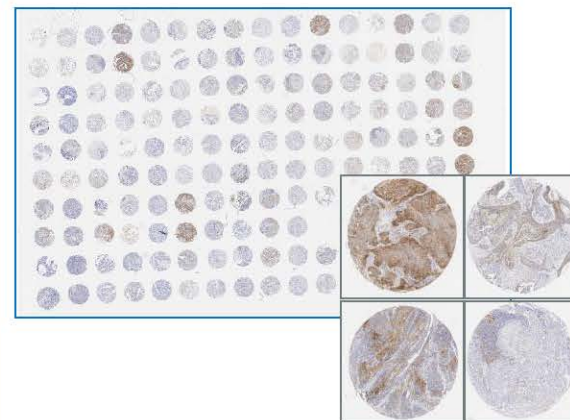


recombinant RabMAb

Recombinant Anti-Uteroglobulin antibody [EPR19846] (ab213203)

Enhanced validation level - extended to relevant tumour tissue

NSCLC TMA



Recombinant anti- PD-L1 (CAL10 ab237726)

Specificity confirmed by KO validation, provides evidence of antibody selectivity for the target of interest only

Neuroscience > Cell Type Marker > Neural Stem Cell marker

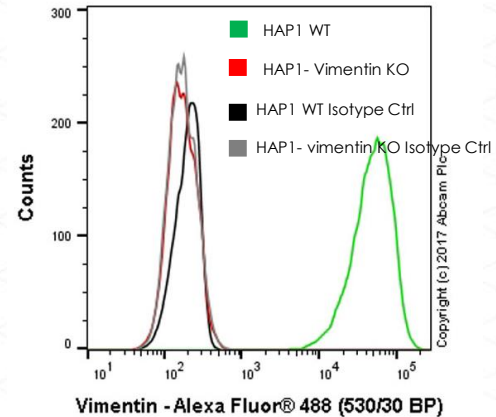
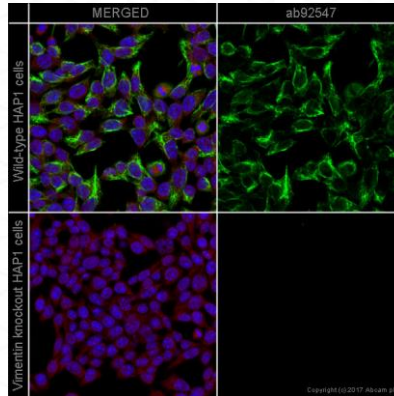
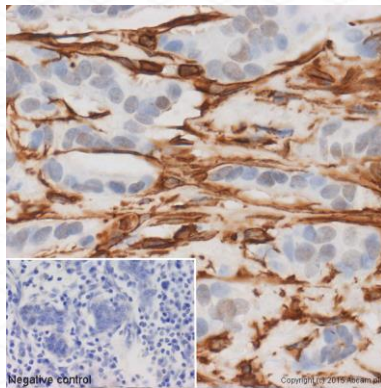
KO VALIDATED Recombinant RabMAb

Share by email

Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

★★★★★ Abreviews (42) Q&A (1) Specific References (235)

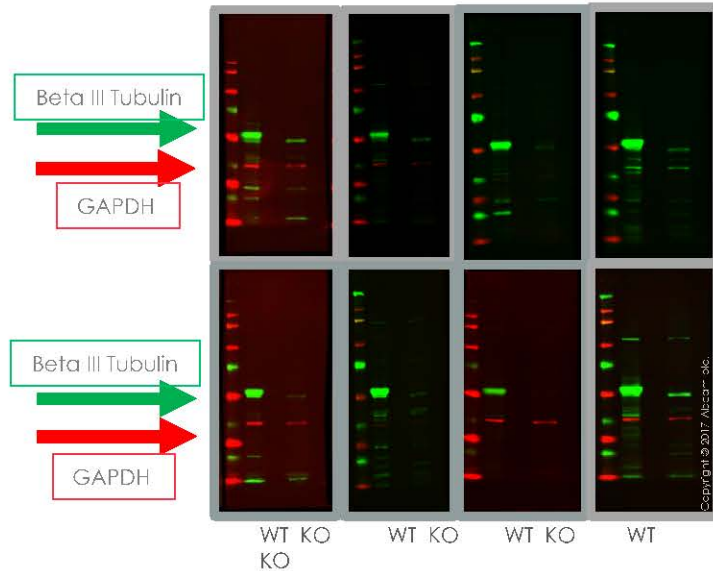
Datasheet SDS



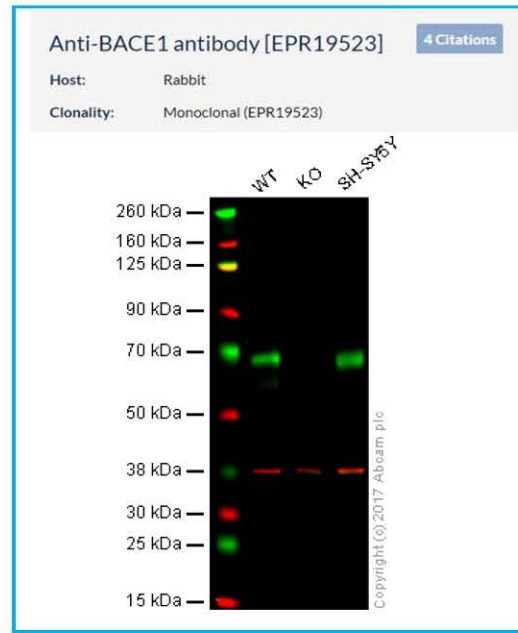
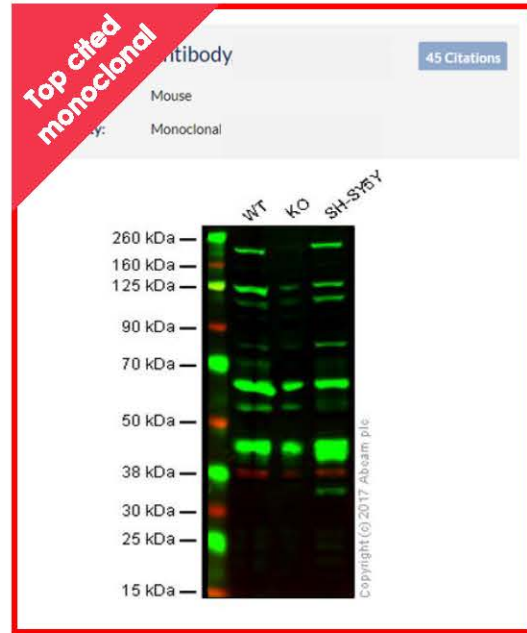
Impact of antibody format on specificity and consistency

Rabbit polyclonal Anti-beta III Tubulin antibody (ab18207)

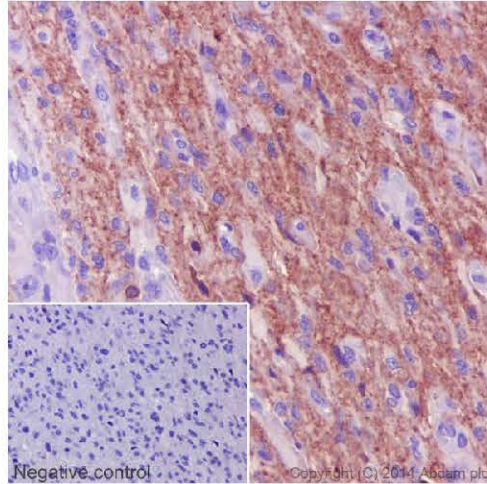
Recombinant Anti-beta III Tubulin antibody [EPR19591] (ab215037)



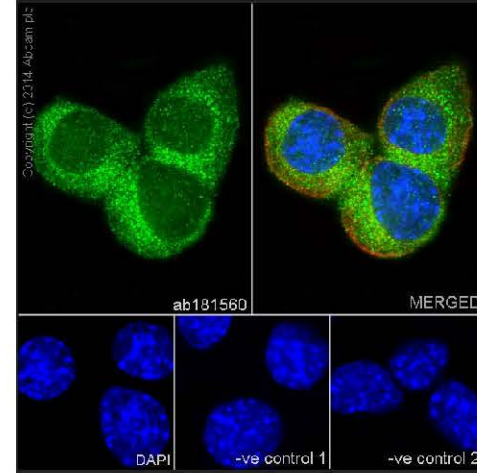
Top cited clones do not always perform the best



Combining recombinant antibody technologies and high validation standards to develop the best reagents



Immunohistochemical analysis of paraffin-embedded Human astrocytoma tissue labelling Pan Trk with ab181560

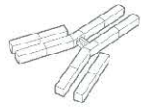


Immunofluorescent analysis of Neuro-2a (Mouse neuroblastoma cells) cells labelling Pan Trk with ab181560

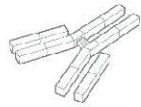
Pan-Trk immunohistochemistry is an efficient and reliable screen for NTRK fusions

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

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



Rapid RabMAb®



B Cell Cloning



NGS

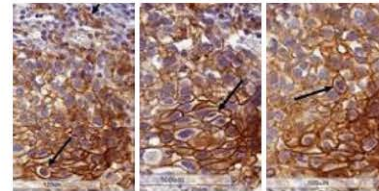
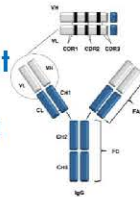


Phage display

... improved sensitivity, specificity and reproducibility

Over 18,000
recombinant
RabMAbs

**Recombinant
rabbit
monoclonal
antibody**



Batch - batch consistency

Questions?
