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.
Advantages and constraints of polyclonal antibodies

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

**Constraints**
- 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

Constraints
- 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

1. Isolation of circulating B cells
2. Isolation and immortalization of B cells
3. NGS
4. Hybridoma derived monoclonal antibody

B) Recombinant monoclonal antibody development - *in vitro* approach

1. Phage M13
2. AxioMx technology
3. Phage Display
4. Recombinant antibodies
Advantages and constraints of immune-based recombinant antibodies

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"

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 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

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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

RabMAb® discovery

AxioMx™ phage display

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

RabMAb rabbit monoclonal antibodies
• High overall affinity
• Broad diversity of epitope recognition

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

Colored product Substrate

donor acceptor

WB Flow cytometry ICC IHC

250kDa 150kDa 100kDa 75kDa 50kDa 37kDa 25kDa 20kDa 15kDa 10kDa
Recombinant anti-BCMA RabMAb® perform in multiple applications

**Western blot**

- MM cell line U266B1

**Immunoprecipitation**

- Ab245940 anti-BCMA

**Flow Cytometry**
Tailored approach to verify antibody specificity

Enhanced validation level - extended to relevant tumour tissue

IHC validation on multispecies and multi-tissue microarray

Mouse lung

Rat lung

Mouse cerebrum

NSCLC TMA

Recombinant Anti-Uteroglobin antibody (EPR19846) (ab213203)

Recombinant anti-PD-L1 (CAL10 ab237726)
Specificity confirmed by KO validation, provides evidence of antibody selectivity for the target of interest only
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

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 rabbit monoclonal antibodies

Batch - batch consistency
Questions?