RabMAbs® (Rabbit Monoclonal Antibodies) A Brief History

Weimin Zhu –
Senior VP of Antibody Technology

May 21, 2013
Topics

Why Rabbit?

History of RabMAB® Technology

Benefits of RabMABs®

Summary
<table>
<thead>
<tr>
<th>Topics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Why Rabbit?</strong></td>
</tr>
<tr>
<td>History of RabMAb® Technology</td>
</tr>
<tr>
<td>Benefits of RabMAbs®</td>
</tr>
<tr>
<td>Summary</td>
</tr>
</tbody>
</table>
Common Antibody Generation Platforms

<table>
<thead>
<tr>
<th>Rodents</th>
<th>Antibody Display Technologies</th>
<th>Chicken</th>
<th>Goat / Sheep</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Monoclonal Antibody</td>
<td>Phage, Yeast, Mammalian</td>
<td>Polyclonal Monoclonal Antibody (TK–chicken myeloma cells)</td>
<td>Polyclonal Sheep Monoclonal Antibody (hetero-hybridoma)</td>
<td>Polyclonal 90’ Rabbit Monoclonal Antibody (240E-W, rabbit myeloma cells)</td>
</tr>
<tr>
<td>Mouse Monoclonal Antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat Monoclonal Antibody</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
So Why RabMAbs®?

On average RabMAbs have the highest sensitivity and specificity.
### Rabbits

<table>
<thead>
<tr>
<th>Breed</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normand</td>
<td><img src="image1" alt="Normand" /></td>
</tr>
<tr>
<td>Blanc de Hotot</td>
<td><img src="image2" alt="Blanc de Hotot" /></td>
</tr>
<tr>
<td>Lièvre belge</td>
<td><img src="image3" alt="Lièvre belge" /></td>
</tr>
<tr>
<td>Bleu de Vienne</td>
<td><img src="image4" alt="Bleu de Vienne" /></td>
</tr>
<tr>
<td>Argenté de Champagne</td>
<td><img src="image5" alt="Argenté de Champagne" /></td>
</tr>
<tr>
<td>Argenté anglais crème</td>
<td><img src="image6" alt="Argenté anglais crème" /></td>
</tr>
<tr>
<td>Hollandais noir</td>
<td><img src="image7" alt="Hollandais noir" /></td>
</tr>
<tr>
<td>Rex tricolore</td>
<td><img src="image8" alt="Rex tricolore" /></td>
</tr>
<tr>
<td>Sablé des Vosges</td>
<td><img src="image9" alt="Sablé des Vosges" /></td>
</tr>
<tr>
<td>Blane néo-zélandais</td>
<td><img src="image10" alt="Blane néo-zélandais" /></td>
</tr>
<tr>
<td>Rex castor</td>
<td><img src="image11" alt="Rex castor" /></td>
</tr>
<tr>
<td>Chinchilla</td>
<td><img src="image12" alt="Chinchilla" /></td>
</tr>
<tr>
<td>Brun marron de Lorraine</td>
<td><img src="image13" alt="Brun marron de Lorraine" /></td>
</tr>
<tr>
<td>Gris su Bourbonnais</td>
<td><img src="image14" alt="Gris su Bourbonnais" /></td>
</tr>
<tr>
<td>Chamois de Thuringe</td>
<td><img src="image15" alt="Chamois de Thuringe" /></td>
</tr>
<tr>
<td>Fauve de Bourgogne</td>
<td><img src="image16" alt="Fauve de Bourgogne" /></td>
</tr>
<tr>
<td>Géant des Flandres</td>
<td><img src="image17" alt="Géant des Flandres" /></td>
</tr>
<tr>
<td>Papillon anglais de Madagascar</td>
<td><img src="image18" alt="Papillon anglais de Madagascar" /></td>
</tr>
</tbody>
</table>

- Rabbit: Family, Leporidae; Order, Lagomorpha
- Genetically quite distant from rodents, out-bred, MHC loci are more diversified

Discover more at abcam.com
Primary antibody repertoire development

**Pro-B cell**

- $D\!J_H$
germline L

**Pre-B cell**

- $V\!D\!J_H$
germline L

**Small pre-B cell**

- $V\!D\!J_H$
germline L

Gene conversion in appendix and other Gut-Associated Lymphoid Tissues (GALT)

B cell development continues after birth (6-8 weeks)
Secondary antibody repertoire development

- Gene conversion
- Hypermutation
- Longer period of maturation
- Antigen presenting CD1 molecules for lipid / glycolipid (5 in rabbit & 1 in mouse)
Rabbits Can Make Better Antibodies – higher affinity, specificity, and broader epitopes recognition

- Unique B cells development process
- Two affinity maturation mechanisms: gene conversion and hypermutation
- A comprehensive antigen-presenting for lipid or glycolipid
- Unique IgG features
  - only one subtype of IgG, and more S-S bonds
  - greater variation in length and sequence, especially in CDR3
  - L chain contributes more to antibody affinity

Larger B cell repertoire with wider diversity
Broader Epitope Recognition

5 mice and 3 rabbits

Immunized with human fibronectin

Polyclonal sera

Western Analysis

1. Molecular-weight marker
2. Fibronectin
3. Protease digested fibronectin
Broader Epitope Recognition – Less immunodominance

ELISA Titer Testing – (Peptide-KLH Immunization, 4 injections, 3 weeks interval)

- Rabbit: ~30% immune response towards peptide, ~70% to KLH
- Mouse: ~10% immune response towards peptide, ~90% to KLH

Discover more at abcam.com
<table>
<thead>
<tr>
<th>RabMAb vs. Mouse MAb</th>
<th>Rabbit</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B cell development</strong></td>
<td>Appendix / GALT</td>
<td>Hypermutation only</td>
</tr>
<tr>
<td>Antibody maturation</td>
<td>Gene conversion + hypermutation</td>
<td></td>
</tr>
<tr>
<td><strong>Affinity</strong></td>
<td>High affinity, picomolar ($K_D=10^{-12}$) possible</td>
<td>Medium affinity, $K_D$ not often $&lt;10^{-9}$</td>
</tr>
<tr>
<td><strong>Epitope recognition</strong></td>
<td>Recognize more epitopes: zoom in on fine molecular details like modification sites of protein</td>
<td>Usually less epitopes on antigen are recognized</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD1 Isotype</strong></td>
<td>More isotypes of CD1 (antigen presenting molecules for lipid and glycolipid): CD1a1, CD1a2, CD1b, CD1d, CD1e</td>
<td>Only one isotype: CD1d</td>
</tr>
<tr>
<td><strong>Immune response to non-protein antigen</strong></td>
<td>Ability to recognize small molecule and hapten</td>
<td>Relatively difficult</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Both human &amp; rodent cross-reactivity easily achieved; ideal for anti-rodent proteins</td>
<td>Limited, not good for rodent proteins</td>
</tr>
</tbody>
</table>
# History of RabMAb® Technology

## Fusion partner cell line development

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
<th>Key Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>Mouse fusion partner cell</td>
<td>Kohler and Milstein</td>
</tr>
<tr>
<td></td>
<td>Myeloma naturally occurs in mice but NOT in Rabbits</td>
<td>Loyola University, Chicago</td>
</tr>
<tr>
<td>1995</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; Rabbit fusion partner cell from transgenic rabbit</td>
<td>Katherine Knight, Robert Pytela, UCSF</td>
</tr>
<tr>
<td></td>
<td>Improved partner cell</td>
<td>Weimin Zhu, Yaohuang Ke, Epitomics</td>
</tr>
<tr>
<td>2005</td>
<td>Remove endogenous H</td>
<td>Weimin Zhu, Yaohuang Ke, Epitomics</td>
</tr>
<tr>
<td></td>
<td>Remove endogenous L</td>
<td>Weimin Zhu, Yaohuang Ke, Epitomics</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Epitomics</td>
</tr>
</tbody>
</table>

- **1979**: Mouse fusion partner cell by Kohler and Milstein. Myeloma naturally occurs in mice but NOT in Rabbits.

- **1995**: 1<sup>st</sup> Rabbit fusion partner cell from transgenic rabbit, developed by Katherine Knight, Robert Pytela, UCSF.

- **1997**: Improved partner cell by Weimin Zhu, Yaohuang Ke, Epitomics.

- **2005**: Remove endogenous H by Weimin Zhu, Yaohuang Ke, Epitomics.

- **2009**: Remove endogenous L by Weimin Zhu, Yaohuang Ke, Epitomics.

---

**Rabbit Monoclonal Antibodies (RabMAbs®)**
- **Rabbit Polyclonal Antibodies**
- **Mouse Monoclonal Antibodies**

Discover more at abcamm.com
RabMAb® Hybridoma Fusion Technology

Immunized Rabbits → Fusion Partner cells (240E-W2, US patent 7429487) → Hybridoma cells → Hybridomas screened by ELISA for specific antigen recognition → RabMAb® Production

Rabbit IgG

VARIABLE REGIONS

CONSTANT REGIONS

FAB

Hinge

VL

CH1

CH2

CH3

VL

FC

Antigen Binding Site

Antigen Binding Site
A Robust Development and Production System (2002 – 2013)

1. Immunogen Design and Preparation
2. Immunization Design and Antiserum Evaluation
3. RabMAb® Hybridoma Generation (240E-W fusion)
4. Screening Assays and Antibody Engineering
5. RabMAb® Antibody Production

Target Selection & Antibody Application – ultimate use

Germinal centers
Affinity maturation

Antigen-presenting cells
B cells T helper cells

Plasma B cells
Memory B cells

Spleen, lymph nodes
bone marrow, PBMC

| Research               | • ~5,000 RabMAbs released  
|                       | • ~15,000 antigens in pipeline  
| Diagnostics (IHC / IVD)| • ~200 RabMAbs (EP clone) released  
|                       | • 4 FDA approved RabMAbs (Class II, Class III)  
| Thera-peutics         | • 1 Phase I; 2 filing IND; 4 preclinical development  
|                       | • Spun-off in 2010 (Apexigen Inc.)  
| Custom Antibody Service| • > 700 Universities, institutes and companies  
|                       | • > 1,900 Custom RabMAbs developed  

Discover more at abcam.com
<table>
<thead>
<tr>
<th>Product Launch Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single band at appropriate MW in native cell lysate or tissue</td>
</tr>
<tr>
<td>Works at minimum of 1:1,000 in WB</td>
</tr>
<tr>
<td>Every product is tested in 5 applications before launch (WB, IHC, ICC/IF, IP and FACS)</td>
</tr>
<tr>
<td>Every product is tested in Human, Mouse and Rat</td>
</tr>
</tbody>
</table>
Target Antigens - Taking Advantage of RabMABs

**Present**

1. **Epitopes** of protein or DNA / RNA modification sites
2. **Epitopes** of antigen in FFPE tissue for IHC
3. **Epitopes** in mouse or rat samples

**Future**

4. **Epitopes** of non-protein antigens
5. **Epitopes** of conformational structure and protein interactions
## RabMAb® Technology History

<table>
<thead>
<tr>
<th></th>
<th>Key Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1995: 1st Rabbit fusion partner cell → 1997 – 2009: Improved fusion partner cell lines (240E-W1, 2, 3)</td>
</tr>
<tr>
<td>2</td>
<td>Robust RabMAb Antibody Development System</td>
</tr>
</tbody>
</table>
| 3 | RabMAb Antibodies recognized as “best in class” 2002 – 2013  
• Taking advantage of RabMAb Technology  
• ~5,000 RabMAbs for life science research use  
• ~200 RabMAbs for anatomic pathology  
• Custom antibodies  
• Therapeutic |
Topics

Why Rabbit?

History of RabMAb® Technology

Benefits of RabMAbs®

Summary
## Benefits of RabMAb Antibodies

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>High affinity and specificity</td>
</tr>
</tbody>
</table>
| 2 | Broader epitopes recognition to zoom in “small” epitopes:  
  - Post-translational modification sites (e.g. phosphorylation, methylation)  
  - Protein cleavage and mutation sites  
  - Conformational epitopes and protein-protein interactions |
| 3 | Ideal for demanding application such as IHC on FFPE tissue |
| 4 | Ideal for studying rodent models |
| 5 | Ideal for antibody against non-protein antigens: lipid, carbohydrate and glycolipid |
| 6 | Ideal for antibody pairing and multiplex immunoassay |
10 – 100 Times Higher Affinity than Mouse MAbs

Antibody Binding Affinity (K_D)

Mouse*  Rabbit MAbs**

* Partial literature survey
** In-house K_D measurements

Learn more at www.abcam.com/KD
Comparison of three commercially available anti-HER2 Abs in paraffin embedded Breast Cancer tissue samples

<table>
<thead>
<tr>
<th>Her2 RabMAb</th>
<th>Her2 Rabbit pAb - Vendor D</th>
<th>Her2 Mouse mAb - Vendor C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab Conc. 3 ng/ml</td>
<td>Ab Conc. 20 ng/ml</td>
<td>Ab Conc. 30 ng/ml</td>
</tr>
</tbody>
</table>

- Even at 10x higher concentration the Mouse mAb was still much weaker
- RabMAbs can have even higher affinity than rabbit polyclonals
Higher Specificity

Comparing Specificity of Anti-hId1 Antibodies

<table>
<thead>
<tr>
<th>RabMAb</th>
<th>Commercial Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Id1</td>
</tr>
<tr>
<td>15</td>
<td>Id1</td>
</tr>
</tbody>
</table>

- Breast Cancer – 468, 231, 435
- Melanoma – A2058, A375P, HT144
- Cervical carcinoma – SiHa, Hela

Note: Data from Stanford Univ. Stanley Cohen’s Lab
RabMAbs to Protein Modification Sites

<table>
<thead>
<tr>
<th>RabMAbs</th>
<th>WB Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Phospho (pY1086) ab32086</td>
<td>A431 cell lysate 1:10,000 dilution</td>
</tr>
<tr>
<td>Stat-5 (pY694) ab32364</td>
<td>A431 cell lysate 1:10,000 dilution</td>
</tr>
<tr>
<td>Histone H4 (actyl K5) ab51997</td>
<td>HeLa cell lysate 1:10,000 dilution</td>
</tr>
</tbody>
</table>

1. Untreated
2. Treated with EGF
3. Phosphatase treated

1. Untreated
2. Treated with TSA
RabMAbs to Protein Cleavage Sites

Full-length PARP (116 kD) is cleaved by caspases in apoptosis to yield two cleaved products: 25 kD and 85 kD.
RabMAb to a Protein Conformational Epitope

Note: Data from A-Cube, Inc

Inactive

SYF (WT) Inactive Src

SYF (CA) Active Src

Active

SYF (WT) Inactive Src

SYF (CA) Active Src
**RabMAb to A Non-Protein Antigen – PEG (polyethylene glycol)**

Pairing RabMAbs has higher sensitivity than pairing RabMAb / mouse MAb

**Sandwich ELISA using RabMAb and MAb**

<table>
<thead>
<tr>
<th>PEG-BSA concentration (ng/mL)</th>
<th>Absorbance (405 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1.2</td>
</tr>
<tr>
<td>250</td>
<td>1.1</td>
</tr>
<tr>
<td>62.5</td>
<td>0.8</td>
</tr>
<tr>
<td>15.625</td>
<td>0.6</td>
</tr>
<tr>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Legend*:
- PEG 47/PEG 47
- APG3/PEG 47

Monitor pharmacokinetics of PEG-IgG in Rat with PEG-RabMAb kit

*Graph*:
- IgG-PEG (ng/mL) vs. time (hours)
- Data points for different groups (e.g., Ctrl Rat 1, Rat 4, etc.)
RabMAbs Work in Multiple Applications

**AIF RabMAb (ab32516)**

<table>
<thead>
<tr>
<th>WB and IP</th>
<th>ICC / IF</th>
<th>FACS</th>
<th>IHC on FFPE tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>(kDa)</td>
<td>![Image]</td>
<td>![Graph]</td>
<td>![Image]</td>
</tr>
<tr>
<td>250</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>![Graph]</td>
<td></td>
</tr>
</tbody>
</table>

HeLa cell stained for AIF subcellular location using AIF RabMab. HeLa cells untreated (A) or treated (B) with Staurosporine

Profile of permeabilized K-562 analyzed on a FACS using AIF RabMab

IHC on FFPE Human Breast Cancer tissue using AIF RabMab

**Currently launched Rabbit Monoclonals:**
- ~85% work in 2 or more applications
- ~62% work in 3 or more applications
- Average of 3 applications per product
Topics

Why Rabbit?

History of RabMAb® Technology

Benefits of RabMAbs®

Summary
Summary - Major Advantages of RabMABs®

- Higher Affinity and Specificity
- Develop antibodies against novel epitopes and challenging antigens
- Cross-species recognition
- Fully validated and work in multiple applications
A chapter on RabMAb and Rabbit Hybridoma technology:


RabMAb technology review article:

http://dc.cn.umb-us.com/i/125322/21

RabMAb Advantages and Benefits:

www.abcam.com/RabMAb

More about $K_D$ - Antibody affinity quantification:

www.abcam.com/KD
Weimin Zhu
Co-founder
Senior VP, Antibody Technology
Epitomics, an Abcam company
Email: weiminzhu@epitomics.com
Products for your research
Rabbit monoclonal antibodies

High quality antibodies for a wide range of applications

- High affinity and specificity
- Diverse epitope recognition
- Extensive validation before release – Tested in multiple applications (WB, IHC, ICC/IF, IP, Flow Cyt) and species (Hu, Ms, Rt)
- Over 5000 RabMAbs available – For multiple research areas and pathways
- Guaranteed high quality as part of our Abpromise

Discover more at abcam.com
Rabbit monoclonal antibodies

Download your FREE copy of the Advantages of RabMAbs booklet

- Discover all 8 advantages
- RabMAb vs. Mouse MAb comparison
- RabMAb product highlights
- And much more…

Find out more at: www.abcam.com/RabMAbs
Free T-shirt and Abpoints for a RabMAbs® Abreview®

Offer valid until end of July 2013. Find out more about this offer at www.abcam.com/RabMAbsAbreviews

Abreviews®
Tell other scientists how our antibodies work for you

- Helpful data to assist other customers in product selection
- Key experimental conditions for assay optimization
- Easy submission via simple online form
- Amazon vouchers or discounts on products for published reviews

See www.abcam.com/abreviews for details
Custom RabMAb services

Our custom services include:

- Rabbit monoclonal and rabbit polyclonal antibody generation
- Immunogen design and preparation
- Immunoassay development
- IgG cDNA cloning and scale-up antibody production

PhD-level project managers:

- Project design point of contact
- Provide updates, technical resource

Experience:

- Clients include more than 550 institutions and 150 pharma and biotech companies
- Flexible payment scheduling and licensing terms

More info on Custom Service:

www.abcam.com/customservices

Discover more at abcam.com
IHC kits and reagents

A comprehensive range which includes

- **EXPOSE IHC kits** – Micro-polymer detection kits offering greater sensitivity than polymer detection

- **Biotin/Streptavidin (ABC) kits** – For established detection methods

- **IHC reagents** – Including blocking sera, tissue pre-treatments and chromogens

Optimized reagents for IHC and an application guide to help with your experimental planning. Find out more at: [www.abcam.com/IHC](http://www.abcam.com/IHC)
Download a tips booklet and Discover reagents to optimize your western blots

- Long shelf life gels
- Optiblot Blue
- Luminol Membrane Pen
- Transfer Membranes
- High sensitivity ECL kits
- Fluorescent detection reagents
- And much more...

Find out more at: www.abcam.com/Optiblot
EasyLink Antibody Conjugation Kits

The easiest way to conjugate your antibodies

• A choice of 18 fluorescent labels
• Rapid labelling protocol
• Available in convenient sizes
  – 3x10µg
  – 1x100µg
  – 3x100µg
  – 1x1mg

Find out more at: http://www.abcam.com/EasyLink
Benefits of EasyLink

Eliminates

- Indirect detection methods
- Column separation steps
- Loss of material
- Non-specific binding of secondary reagents
- Additional incubation steps and sample dilutions

How the kit works

1. Add EL-Modifier
2. Add antibody solution to EL-Conjugate
3. Incubate for 3h RT
4. Add EL-Quencher for 30min
5. Ready to use
Brand new – Alexa Fluor® conjugated secondary antibody range

Alexa Fluor® 488  Alexa Fluor® 555  Alexa Fluor® 594  Alexa Fluor® 647

www.abcam.com/alexa

Alexa Fluor® is a registered trademark of Life Technologies. Alexa Fluor® dye conjugates contain(s) technology licensed to Abcam by Life Technologies.
Why to choose an Abcam Alexa Fluor® conjugated secondary?

- Extensively tested in the Abcam laboratories – To guarantee bright staining and low background
- Large selection of pre-adsorbed antibodies-ensuring low species cross-reactivity
- Dilution range of 1/200 – 1/1000 (10-2 μg/ml) in IF/ICC – Perform at least 250 stainings*
- Antibodies conjugated to Alexa Fluor® 488/555/594/647 – For your multi-color imaging experiments
- Competitively priced

www.abcam.com/alexa

Alexa Fluor® is a registered trademark of Life Technologies. Alexa Fluor® dye conjugates contain(s) technology licensed to Abcam by Life Technologies.
Webinar promotion

A small thank you

- Save 25% on RabMAbs, Alexa Fluor® conjugated secondary antibodies, IHC kits and reagents, the Optiblot range and EasyLink Conjugation Kits
- You will receive the promo code in an email after the webinar
- This promotion is valid till end of June

Alexa Fluor® is a registered trademark of Life Technologies. Alexa Fluor® dye conjugates contain(s) technology licensed to Abcam by Life Technologies.
Questions?