RabMAb® advantages
A guide to our rabbit monoclonal antibodies

Discover more at abcam.com/RabMAb
“The RabMAb® products I received are specific to an immunogen with no crossreactivity to other peptides or proteins, and possess higher affinity than mouse monoclonal antibodies.”

Dr. Pankaj D. Mehta, Institute for Basic Research in Developmental Disabilities (NY)
About RabMAb® Technology

RabMAb® technology is a unique and proprietary method for developing monoclonal antibodies (MAbs) from rabbits rather than the conventional method of using mice (Figure 1). This delivers antibodies with the high affinity of a rabbit polyclonal and the high specificity of a monoclonal.

RabMAb® antibodies provide better antigen recognition than traditional murine antibodies\(^1,2\) due to the unique rabbit immune system. Compared to mice and other rodents, rabbits:

- Mount immune responses against a broader range of compounds, including various murine proteins
- Lower immune dominance towards carrier proteins
- Undergo more somatic gene conversion
- Possess longer and more heterogeneous CDR3 sequences for increased sequence variation

The combination of these properties results in a wider range of antibodies and therefore a better chance of obtaining a functional antibody that will work in a variety of applications\(^3\).


## RabMAb® technology vs traditional mouse monoclonal technology

<table>
<thead>
<tr>
<th>Comparison</th>
<th>RabMAb® technology</th>
<th>Mouse MAb technology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANTIGEN RECOGNITION</strong></td>
<td>- High chance of success with a range of antigens, including small molecules and peptides</td>
<td>- Small molecules and peptides often non-immunogenic</td>
</tr>
<tr>
<td></td>
<td>- Good responses to rodent proteins</td>
<td>- Very limited response to rodent antigens</td>
</tr>
<tr>
<td></td>
<td>- Antibodies recognize several epitopes per protein antigen</td>
<td>- Antibodies recognize a limited number of epitopes (due to immunodominance)</td>
</tr>
<tr>
<td><strong>AFFINITY</strong></td>
<td>Picomolar ($10^{-12}$ K$_D$ M) possible</td>
<td>Nanomolar ($10^{-9}$ K$_D$ M)</td>
</tr>
<tr>
<td><strong>SPECIFICITY</strong></td>
<td>High</td>
<td>Medium/high</td>
</tr>
<tr>
<td><strong>APPLICATIONS</strong></td>
<td>Western blot, ELISA, flow cytometry, IP, IHC, ICC (excellent results in IHC)</td>
<td>Western blot, ELISA, flow cytometry, IP, not always suitable for IHC and ICC</td>
</tr>
</tbody>
</table>

### CDX2
Paraffin-embedded human colonic carcinoma tissue stained with CDX2 rabbit monoclonal (ab76541) and a competitor’s CDX2 mouse monoclonal (both at 1:1000 dilution factor)

![RabMAb® Primary Antibody](image1)

![Mouse MAb](image2)

### E-CADHERIN
Paraffin-embedded human breast carcinoma tissue stained with E-Cadherin rabbit monoclonal (ab40772) and a competitor’s E-Cadherin mouse monoclonal (both at 1:50 dilution factor)

![RabMAb® Primary Antibody](image3)

![Mouse MAb](image4)

### HER2/ErbB2
Paraffin-embedded human breast carcinoma tissue stained with HER2/ErbB2 rabbit monoclonal (ab134182) and a competitor’s HER2/ErbB2 mouse monoclonal (both at 1:500 dilution factor)

![RabMAb® Primary Antibody](image5)

![Mouse MAb](image6)
What are the RabMAb® advantages?

RabMAb® antibodies provide the combined benefits of superior antigen recognition of the rabbit immune system with the specificity and consistency of a monoclonal antibody, bringing you the highest quality antibody possible.

What do these advantages mean to you?

High-quality antibodies for a wide range of applications

RabMAb® primary antibodies are ideal for most demanding applications such as IHC on formalin-fixed, paraffin-embedded (FFPE) tissue.

Novel antibodies for previously hard-to-generate targets

Highly functional RabMAb® antibodies to novel targets can be developed. One example is development of high-quality antibodies to dual phosphorylated sites.

Extensive validation for antibodies you can trust

Every RabMAb® primary antibody is screened and tested in multiple applications (ELISA, WB, IHC, ICC/IF, IP and FCM) and species (human, mouse and rat) to ensure the highest quality possible.
1. Low background

RabMAb® primary antibodies typically provide more specific and sensitive detection of their target protein with low background.

As monoclonals, RabMAb® primary antibodies detect a single epitope and are less likely to cross-react with other proteins. At the same time, RabMAb® primary antibodies bind to their target with greater affinity, enabling higher signal to-noise ratio than mouse monoclonal antibodies at a given antibody concentration.

**Figure 2. Specific and sensitive detection of the target protein**

<table>
<thead>
<tr>
<th>c-Myc</th>
<th>AMPK alpha 1 (phospho S496)</th>
<th>MCM2</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Histone H3 (phospho S10)</th>
<th>ERG</th>
<th>Ki67 (Alexa Fluor® 647)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF on HeLa using anti-Histone H3 (phospho S10) rabbit monoclonal (ab32107).</td>
<td>IHC on human prostate cancer tissue on anti-ERG rabbit monoclonal (ab92513).</td>
<td>Flow cytometry on HeLa cells using anti Ki67 (Alexa Fluor 647, ab194724, red), rabbit IgG isotype control (black), unlabeled sample (blue).</td>
</tr>
</tbody>
</table>
2. Ideal for detecting post-translational modifications

The ability of rabbit generated antibodies to recognize small epitopes translates to success with recognition of post-translational modifications (eg phosphorylation, methylation, acetylation, sumoylation).

Figure 3. Examples of histone H3 modification-specific RabMAb® primary antibodies

<table>
<thead>
<tr>
<th>Acetylation</th>
<th>Methylation</th>
<th>Phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="" /></td>
<td><img src="image" alt="" /></td>
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</tr>
</tbody>
</table>

WB on C6 cell lysates using anti-histone H3 (acetyl K56) rabbit monoclonal (ab76307). Lane 1: untreated, lane 2: treated with TSA.

WB using anti-histone H3 (di methyl K4) rabbit monoclonal (ab32356). Lane 1: HeLa cell lysate, lane 2: recombinant histone H3 (non-methylated).

WB on NIH 3T3 cell lysates using anti-Histone H3 (phospho S28) rabbit monoclonal (ab32388). Lane 1: untreated, lane 2: treated with FBS and Calyculin A.

Figure 4. Examples of phospho site-specific RabMAb® primary antibodies

<table>
<thead>
<tr>
<th>Phosphotyrosine (pY)</th>
<th>Phosphoserine (pS)</th>
<th>Phosphothreonine (pT)</th>
<th>Dual Phospho (pT/pS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="" /></td>
<td><img src="image" alt="" /></td>
<td><img src="image" alt="" /></td>
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</tr>
</tbody>
</table>

WB on A431 cell lysates using anti-phospho-EGFR (phospho Y1110) rabbit monoclonal (ab68470). Lane 1: untreated, lane 2: treated with EGF.

WB on NIH 3T3 cell lysate using anti-c-Jun (phospho S63) rabbit monoclonal (ab32385). Lanes 1 and 3: untreated, lanes 2 and 4: treated with UV or anisomycin.

WB on 293 cell lysate using anti-elf4EBP1 (phospho T37) rabbit monoclonal (ab75767). Lane 1: untreated, lane 2: treated with insulin.

WB on 293T cell lysate using anti-phospho-S6K (pT421/pS424) rabbit monoclonal (ab32525). Lane 1: untreated, lane 2: treated with EGF.
3. Excellent for IHC

RabMAb® technology offers increased sensitivity with no loss of specificity, making antibodies ideal for demanding applications like IHC on FFPE tissues. RabMAb® primary antibodies permit higher working dilutions (5 - 10X on average) and can be used with various tissue fixations, such as FFPE at minimal level of pretreatment. Additionally, when used along with a mouse monoclonal, one can perform dual staining with two monoclonal antibodies for high quality double staining on the same tissue sample.

Figure 5. HER2 RabMAb® primary antibody IHC comparison

<table>
<thead>
<tr>
<th>HER/ErbB2 RabMAb® primary antibody</th>
<th>Rabbit polyclonal antibody (Vendor A)</th>
<th>Mouse monoclonal antibody (Vendor B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ng/mL</td>
<td>20 ng/mL</td>
<td>30 ng/mL</td>
</tr>
</tbody>
</table>

A comparison of HER2/ErbB2 rabbit monoclonal against leading commercially available HER2/ErbB2 rabbit polyclonal (Vendor A) and mouse monoclonal (Vendor B) on FFPE human breast carcinoma tissue. Recommended IHC protocol and dilution factor were used for each case, the antibody concentration used listed below for each stain.

Figure 6. Examples of double staining using RabMAb® primary antibodies

- IHC on human urinary bladder cancer tissue using anti-CK8 rabbit monoclonal (ab53280) with Fast Red and anti-Ki67 mouse monoclonal with DAB.
- IHC on human breast cancer tissue using anti-HER2/ErbB2 rabbit monoclonal (ab134182) with Fast Red and anti-ER alpha mouse monoclonal with DAB.
- IHC on human cervical carcinoma tissue using anti-AIF rabbit monoclonal (ab32516) with Fast Red and anti-Ki67 mouse monoclonal with DAB.
4. High affinity

Antibody affinity is typically represented by the equilibrium dissociation constant ($K_D$), a ratio of $k_{off}/k_{on}$ between the antibody and its antigen, where lower $K_D$ value suggests higher affinity relationship\(^1\).

While most therapeutic monoclonal antibodies have a $K_D$ value in the nanomolar range ($K_D = 10^{-9}$ M), RabMAb® primary antibodies consistently demonstrate higher affinity. With the $K_D$ values which can often reach the picomolar level ($K_D = 10^{-12}$ M), effectively eliminating the need for further affinity maturation\(^2\).

**Affinity comparison by $K_D$ value against popular therapeutic antibodies**

<table>
<thead>
<tr>
<th>RabMAb® primary antibody</th>
<th>$K_D$ (M)</th>
<th>Mouse Mab</th>
<th>$K_D$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>$1.28 \times 10^{-12}$</td>
<td>Herceptin</td>
<td>$5.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>ID1</td>
<td>$2.82 \times 10^{-12}$</td>
<td>Rituxan</td>
<td>$8.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>C29</td>
<td>$9.57 \times 10^{-11}$</td>
<td>Synagis</td>
<td>$1.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>$1.25 \times 10^{-11}$</td>
<td>Remicade</td>
<td>$2.0 \times 10^{-10}$</td>
</tr>
<tr>
<td>IL-1-beta</td>
<td>$1.99 \times 10^{-10}$</td>
<td>Avastin</td>
<td>$5.0 \times 10^{-10}$</td>
</tr>
</tbody>
</table>

**Figure 7. Affinity meter: RabMAb® primary antibodies vs typical monoclonal antibodies**

Antibody affinity comparison by KD value for RabMAb® primary antibodies vs. typical Mabs. Measurement based on $K_D$ measurement analysis of over 850 RabMAb® primary antibodies. RabMAb® primary antibody displays antibody affinity level in the picomolar range compare to typical MAbs which are in the nano molar range.


5. High specificity

RabMAb® antibodies are highly specific and able to distinguish between very similar proteins or sequences. While mouse MAbs can also be highly specific, they often cannot recognize subtle changes in epitopes.

The example below demonstrates the high specificity of an anti-progesterone RabMAb® primary antibody, that does not cross-react with closely related analogues of progesterone.

<table>
<thead>
<tr>
<th>Identity</th>
<th>% Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>100</td>
</tr>
<tr>
<td>17α-Hydroxyprogesterone</td>
<td>0.901</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.035</td>
</tr>
<tr>
<td>Desoxycorticosterone</td>
<td>1.59</td>
</tr>
<tr>
<td>20α Dihydroprogesterone</td>
<td>0.016</td>
</tr>
<tr>
<td>20β Dihydroprogesterone</td>
<td>0.02</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>0.764</td>
</tr>
<tr>
<td>Pregnenolone-3-SO₄</td>
<td>0.426</td>
</tr>
</tbody>
</table>

RabMAb® technology delivers superior specificity for the generation of antibodies that recognize subtle changes in epitopes, such as those created upon protein cleavage.

**Figure 8. Specificity of PARP-1 RabMAb® primary antibodies**

A comparison of poly (ADP-ribose) polymerase 1 (PARP-1)-specific RabMAb® antibodies. Each antibody specifically recognizes a cleavage site, with no-cross-reactivity to non-specific cleavage sites.
6. Recognition of diverse and novel epitopes

Compared with the mouse immune system, the rabbit immune system has lower immune dominance and larger B-cell repertoire. The benefit is a wider epitope recognition during antibody development.

The comparison of rabbit and murine immune responses below shows that the rabbit antisera recognizes a wider range of epitopes than the mouse by western blot analysis. Our experience in antibody development has demonstrated that the rabbit immune system will generally yield a wider range of antibodies recognizing unique epitopes.

**Figure 9. Immune response comparison: rabbit vs mouse**

Rabbit antisera recognizes a wider range of epitopes than the mouse.

In addition, many major relevant human protein targets are highly conserved between mouse and human. Therefore, these proteins tend to be less immunogenic when using mouse or rat as a host. With its unique mechanism of immune diversification and affinity maturation, rabbit antibodies can be produced to a wider range of epitopes to enable the discovery of more novel antibody targets.
7. Fully validated in multiple applications

Every RabMAb® primary antibody is tested in multiple applications (WB, IHC, ICC/IF, IF, IP and FCM) and multiple species (human, mouse and rat) before release. For IHC, each RabMAb® primary antibody is tested on multiple FFPE human tissue array for more accurate verification of antibody sensitivity and localization.

**Figure 10. Validation results for Caspase-3 (Pro) RabMAb® primary antibody**

![Validation data for Caspase-3 (Pro) rabbit monoclonal (ab32150) on A) WB on HeLa cell lysate, B) IHC on FFPE human cervical carcinoma tissue, C) FCM on Jurkat cells and D) ICC on HeLa cells.](image)

“We have spent the last year buying AGR2 antibody from various companies and sometimes from the same company with different lot numbers, searching for an antibody that does not cross-react with AGR3 and therefore is specific for AGR2, does not give us background when developing western blots, does not give us background in negative control when looking at IF staining in tissue, and of course works well for both western blot and IHC.

**We have finally found it with AGR2 RabMAb® product from Abcam!**

Researcher, University of Southern California
8. Ideal for use on mouse samples

Use of mouse MAbs on mouse tissues can be problematic and require complex protocols due to cross-reactivity and high background from anti-mouse secondary antibodies. With the rabbit as a host, RabMAb® technology can produce a monoclonal antibody to mouse targets which are less immunogenic in mouse or rat.

With RabMAb® primary antibodies, you can use monoclonal antibodies in mouse models without the issue of nonspecific signal due to native mouse Ig cross-reactivity.

Every RabMAb® primary antibody has been tested for species cross-reactivity in western blot before release, using both human and mouse samples.

**Figure 11. RabMAb® validation on mouse tissue by IHC**

- **EGFR Phospho (pY1092)**
  - IHC on FFPE mouse intestine using anti-EGFR (phospho Y1092) rabbit monoclonal (ab40815).

- **Cytokeratin 8**
  - IHC on FFPE mouse colon using anti-Cytokeratin 8 rabbit monoclonal (ab53280).

- **MCM2**
  - IHC on FFPE mouse intestine using anti-MCM2 rabbit monoclonal (ab108935).

- **GFAP**
  - IHC on FFPE mouse brain using anti-GFAP rabbit monoclonal (ab68428).

- **CD3 gamma**
  - IHC on FFPE mouse spleen using anti-CD3 gamma rabbit monoclonal (ab134096).

- **p120 Catenin**
  - IHC on FFPE mouse large intestine using anti-p120 Catenin rabbit monoclonal (ab92514).
Featured RabMAb® Products

NeuN antibody (EPR12763) (ab177487)

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>Hu, Ms, Rt, Sh, Gt, Ct, Dg, Zf, Mtt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>WB, ICC/IF, IHC-FoFr, IHC-Fr, IHC-P</td>
</tr>
<tr>
<td>Amount</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

ATM (phospho S1981) antibody (EP1890Y) (ab81292)

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>Hu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>WB, IHC-P, ICC/IF, IP</td>
</tr>
<tr>
<td>Amount</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

beta Catenin antibody (E247) (ab32572)

<table>
<thead>
<tr>
<th>Reactivity</th>
<th>Hu, Ms, Rt, Hm, Mk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>WB, IHC-F, IHC-P, ICC/IF, IP</td>
</tr>
<tr>
<td>Amount</td>
<td>100 µl</td>
</tr>
</tbody>
</table>
c-Myc antibody (Y69) (ab32072)

**Reactivity:** Hu, Ms, Rt  
**Applications:** WB, IHC-P, ICC/IF, IP  
**Amount:** 100 µl

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EGFR (phospho Y1092) antibody (EP774Y) (ab40815)

**Reactivity:** Hu, Ms  
**Applications:** WB, IHC-F, IHC-P, ICC/IF  
**Amount:** 100 µl

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alpha Tubulin antibody (EP1332Y) (ab52866)

**Reactivity:** Hu, Ms, Rt  
**Applications:** WB, IHC-F, IHC-P, ICC/IF, IP, FCM  
**Amount:** 100 µl
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