Advantages of RabMAbs®

abcam®
discover more

Rabbit Monoclonal Antibodies (RabMAbs®) offer multiple advantages to bring you the highest quality antibody possible.

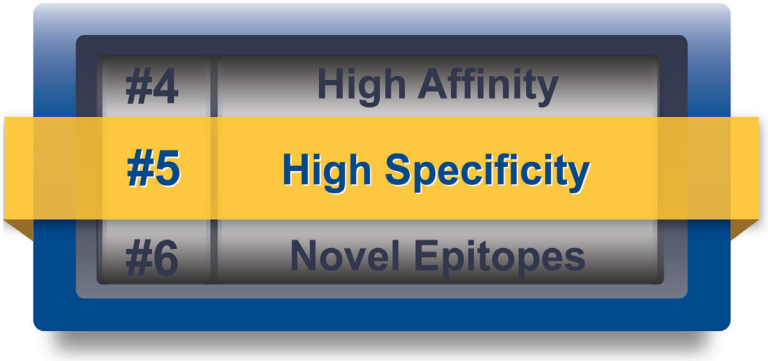


Table of Contents

RabMAbs offer multiple advantages over the traditional mouse monoclonal and rabbit polyclonal antibodies. Here, we highlight 8 unique advantages of RabMAbs:

RabMAb Advantages

About RabMAb Technology

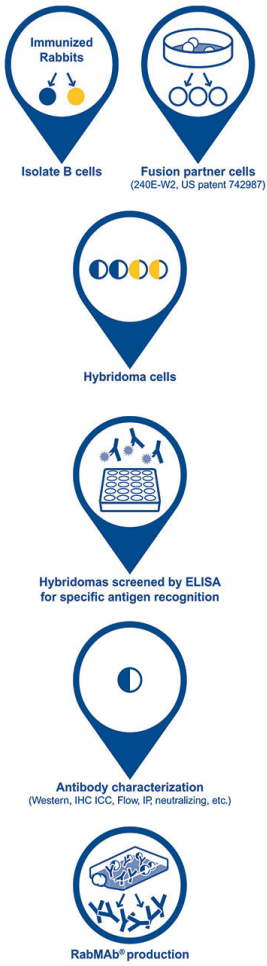
Rabbit Monoclonal Antibodies (RabMAbs®) are developed using a unique and proprietary method for developing monoclonal antibodies from rabbits rather than the conventional method of starting with mice.

The basic principle for making RabMAbs is the same as for mouse monoclonals. Proprietary rabbit fusion partner cells can fuse to rabbit B-cells to create the Rabbit Hybridoma cells. Hybridomas are then screened to select for clones with specific and sensitive antigen recognition and the antibodies are characterized using a variety of methods (Fig. 1).

Antibodies generated with RabMAb technology provide a better antigen recognition in comparison with traditional murine antibodies. The reason for this is that the rabbit immune system generates antibody diversity and optimizes affinity by mechanisms that are more efficient than those of mice and other rodents. This increases the possibility of obtaining a functional antibody that will work in a variety of applications (1, 2).

Additionally, many small compounds and peptides do not elicit a good immune response in mice but do so in rabbits. It is often for this reason, that rabbit polyclonal antibodies are developed and used in many research and diagnostic applications, ranging from drug screening to clinical diagnosis.

Fig. 1 - An overview of RabMAb development



1. Rabbit monoclonal antibodies: a comparative study between a novel category of immunoreagents and the corresponding mouse monoclonal antibodies.

Rossi S, [*Am J Clin Pathol.* 2005 Aug;124(2):295-302]

2. Rabbit monoclonal antibodies show higher sensitivity than mouse monoclonals for estrogen and progesterone receptor evaluation in breast cancer by immunohistochemistry. Rocha R, [*Pathol Res Pract*, 2008;204(9):655-62. Epub 2008 Jun 18]

What are the RabMAb advantages?

Rabbit Monoclonal Antibodies (RabMAbs®) 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.

Published results from independent laboratories comparing rabbit monoclonal and mouse monoclonal antibodies have found RabMAbs to offer increased sensitivity with similar or better specificity than comparable mouse monoclonal antibodies.

Some unique benefits of RabMAbs:

- Diverse epitope recognition
- Improved immune response to small-size epitopes
- High specificity and affinity
- Improved response to mouse antigens

What do these advantages mean to you?

High quality antibodies for a wide range of applications

The rabbit immune system generates antibody diversity and optimizes affinity by mechanisms that are more efficient than those of mice and other rodents. This increases the possibility of obtaining a functional antibody that will work in a variety of applications. RabMAbs are even ideal for most demanding applications such as IHC on formalin-fixed, paraffin-embedded (FFPE) tissue.

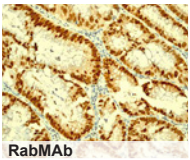
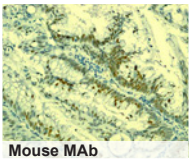
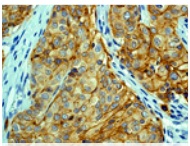
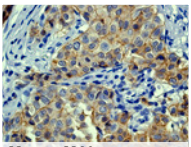
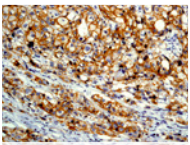
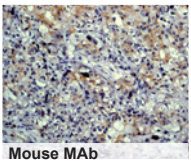
Novel antibodies for previously hard-to-generate targets

With a RabMAb's diverse epitope recognition and improved immune response to non-immunogenic targets (e.g. mouse antigens, small-size epitopes, small compound/peptides), allows for development of highly functional antibodies to novel targets. One example is development of high quality antibodies to dual phosphorylated sites.

Extensive validation

In addition to the benefits of the rabbit monoclonal technology, every RabMAb is screened and tested in multiple applications (ELISA, WB, IHC, ICC/IF, IP and FACS) and species (Human, Mouse, Rat). Extensive validation and Abcam's standard for high quality products, equates to **the highest quality antibody possible**.

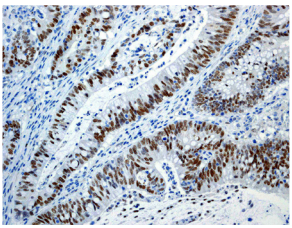
RabMAbs comparison with Mouse Monoclonal Antibodies

Comparison	RabMAbs	Mouse monoclonal
ANTIGEN RECOGNITION	• High chance of success with range of antigens including small molecules and peptides • Good responses to rodent proteins • Recognizes several epitopes per protein antigen	• Limited immuno response • Small molecules/peptides often non-immunogenic • Very limited response to rodent antigens • Recognizes limited number of epitopes (due to immuno-dominance)
AFFINITY	Picomolar (10^{-12} K_D M) possible	Nanomolar (~10^{-9} K_D M)
SPECIFICITY	High	Med-High
APPLICATIONS	Western blot, ELISA, Flow Cytometry, IP, IHC, ICC (excellent results in IHC)	Western blot, ELISA, Flow Cytometry, IP, not always suitable for IHC, ICC.
CDX2 Paraffin-embedded human colonic carcinoma tissue stained with CDX2 RabMAb (ab76541) and Vendor A's CDX2 mouse monoclonal (both at 1:1000 dilution factor)		
E-CADHERIN Paraffin-embedded human breast carcinoma tissue stained with E-Cadherin RabMAb (ab40772) and Vendor A's E-Cadherin mouse monoclonal (both at 1:50 dilution factor)		
HER2 / ErbB2 Paraffin-embedded human breast carcinoma tissue stained with HER2 / ErbB2 RabMAb (ab134182) and Vendor A's HER2 / ErbB2 mouse monoclonal (both at 1:500 dilution factor)		

1. Low background

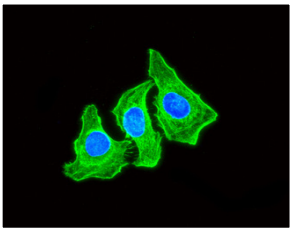
Being monoclonals, RabMAbs detect a single epitope and are therefore less likely to cross-react with other proteins. At the same time we have observed that RabMAbs bind to their target with greater affinity enabling higher signal-to-noise ratio than mouse mAbs at a given concentration of antibody. The benefit of this is that RabMAbs typically provide more specific and sensitive detection of their target protein with low background.

c-Myc



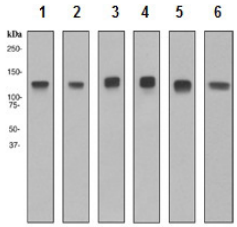
IHC on FFPE human colonic adenocarcinoma using anti-c-Myc RabMAb (ab32072).

AMPK alpha 1 (phospho S496)



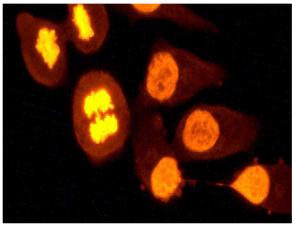
IF on HeLa cells using anti-Phospho-AMPK alpha 1 (pS496) RabMAb (ab92701).

MCM2



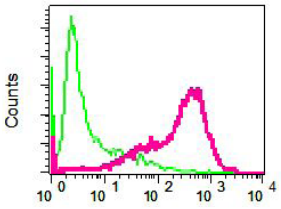
WB on (1) MCF-7, (2) Ramos, (3) SW480, (4) Molt-4, (5) Jurkat, and (6) HeLa cell lysates using anti-MCM2 RabMAb (ab108935).

Histone H3 (phospho S10)



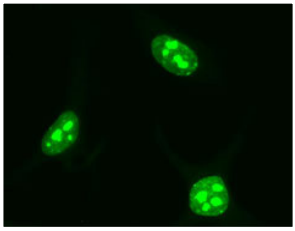
IF on HeLa using anti-Histone H3 (phospho S10) RabMAb (ab32107).

CD38



FACS on HuT-78 cells using anti-CD38 RabMAb (red) (ab108403) and a rabbit IgG (negative) (green).

Ki67 (Alexa Fluor® 488)



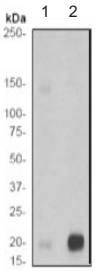
IF on HeLa cells using anti-Ki67 RabMAb (ab154201).

2. Ideal for post-translational modification detection

The ability of rabbit generated antibodies to recognize small epitopes translates to success with recognition of post-translational modifications (e.g. phosphorylation, methylation, acetylation, sumoylation). In addition, many small compounds and peptides do not elicit a good immune response in mice but do so in rabbits.

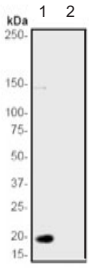
Examples of Histone H3 modification specific RabMAbs

Acetylation



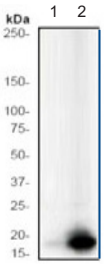
WB on C6 cell lysates using anti-Histone H3 (acetyl K56) RabMAb (ab76307). Lane 1: untreated, lane 2: treated with TSA.

Methylation



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

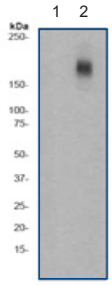
Phosphorylation



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

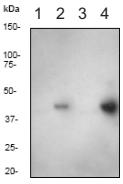
Examples of RabMAbs detecting specific phospho sites

Phosphotyrosine (pY)



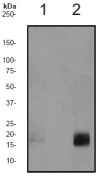
WB on A431 cell lysates using anti-Phospho-EGFR (Phospho Y1110) RabMAb (ab68470). Lane 1: untreated, lane 2: treated with EGF.

Phosphoserine (pS)



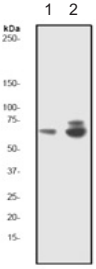
WB on NIH 3T3 cell lysate using Anti-c-Jun (phospho S63) RabMAb (ab32385). Lanes 1 and 3: untreated, lanes 2 and 4: treated with UV or Anisomycin.

Phosphothreonine (pT)



WB on Jurkat cell lysate using anti-eIF4EBP1 (phospho T37) RabMAb (ab75767). Lane A1 untreated, lane 2: treated with insulin.

Dual Phospho (pT/pS)

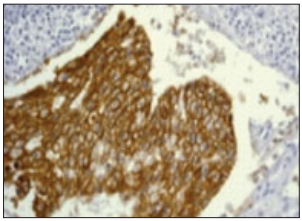
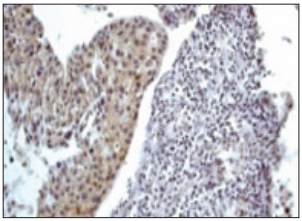
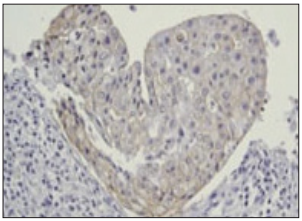


WB on 293T cell lysate using anti-Phospho-S6K (pT421/ pS424) RabMAb (ab32525). Lane 1: untreated, lane 2: treated with EGF.

3. Excellent for IHC usage

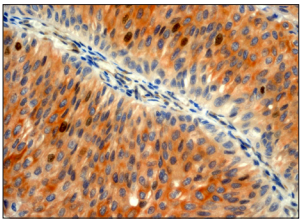
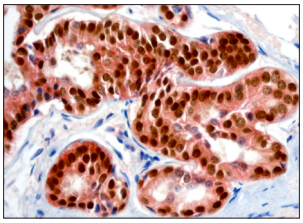
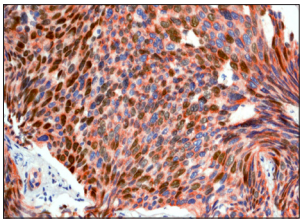
RabMAbs offer increased sensitivity with no loss of specificity, making them ideal for demanding applications like IHC on FFPE tissues. RabMAbs permit higher working dilutions (5 - 10X on average) and can be used with various tissue fixations, such as FFPE at minimal level of pretreatment. Additionally, 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.

HER2 RabMAb IHC comparison

HER2 / ErbB2 RabMAb	Rabbit Polyclonal Antibody (Vendor A)	Mouse Monoclonal Antibody (Vendor B)
		
3 ng / mL	20 ng / mL	30 ng / mL

A comparison of HER2 / ErbB2 RabMAb 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.

Examples of double staining using RabMAbs

CK8 & Ki67	HER2 / ErbB2 & ER Alpha	AIF & Ki67
		
IHC on human urinary bladder cancer tissue using anti-CK8 RabMAb (ab53280) with Fast Red and anti-Ki67 Mouse MAb with DAB.	IHC on human breast cancer tissue using anti-HER2 / ErbB2 RabMAb (ab134182) with Fast Red and anti-ER Alpha Mouse MAb with DAB.	IHC on human cervical carcinoma tissue using anti-AIF RabMAb (ab32516) with Fast Red and anti-Ki67 Mouse MAb with DAB.

4. High affinity

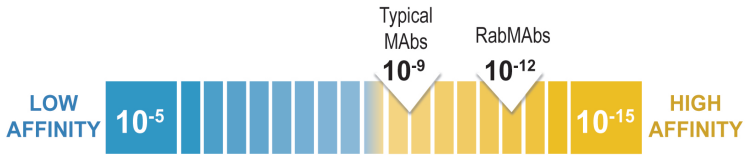
Antibody affinity is typically represented by the equilibrium dissociation constant (K_D), a ratio of k_{off}/k_{on}, between the antibody and its antigen, where lower K_D value suggests higher affinity relationship (1). While most therapeutic monoclonal antibodies have a K_D value in the nanomolar range ($K_D = 10^{-9}$ M), RabMAbs 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 for RabMAbs vs. popular therapeutic antibodies

RabMAb	K_D (M)
ER	1.28×10^{-12}
ID1	2.82×10^{-12}
C29	9.57×10^{-11}
TNF-alpha	1.25×10^{-11}
IL-1-beta	1.99×10^{-10}

Marketed Therapeutic MAb	K_D (M)
Herceptin	5.0×10^{-9}
Rituxan	8.0×10^{-9}
Synagis	1.0×10^{-9}
Remicade	2.0×10^{-10}
Avastin	5.0×10^{-10}

Affinity Meter: RabMAbs vs. Typical MAbs



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

1. Applications And Engineering Of Monoclonal Antibodies. David J. King, 2007.

2. Anti-peptide antibody screening: Selection of high affinity monoclonal reagents by a refined surface plasmon resonance technique. Pope ME, [*J Immunol Methods*. 2009 Feb 28;341(1-2):86-96. doi: 10.1016/j.jim.2008.11.004. Epub 2008 Nov 28]

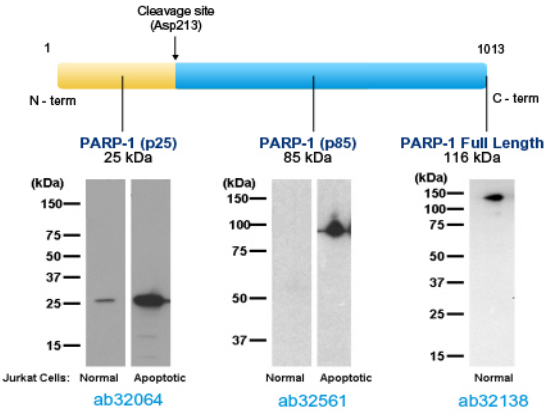
5. High specificity

The RabMAb technology is capable of delivering antibodies that are highly specific and able to distinguish between very similar proteins or sequences. While mouse mAbs can also be highly specific, subtle changes in epitopes are often not recognizable by the mouse immune system. On the contrary, the rabbit immune system is capable of recognizing subtle differences, thus enabling the generation of antibodies for epitopes that distinguish one protein from another. The example below demonstrates the high specificity of anti-Progesterone RabMAb, as it does not cross-react with closely related analogues of progesterone.

Identity	% Reactivity
Progesterone	100
17-aHydroxyprogesterone	0.901
Cortisol	0.035
Desoxycorticosterone	1.59
20a Dihydroprogesterone	0.016
20b Dihydroprogesterone	0.02
Pregnenolone	0.764
Pregnenolone-3-SO_4	0.426

A RabMAb's superior specificity allows for generation of high quality antibodies which recognize subtle changes in epitopes, such as those created upon protein cleavage or between closely related proteins.

A comparison of specificity of various PARP RabMAbs

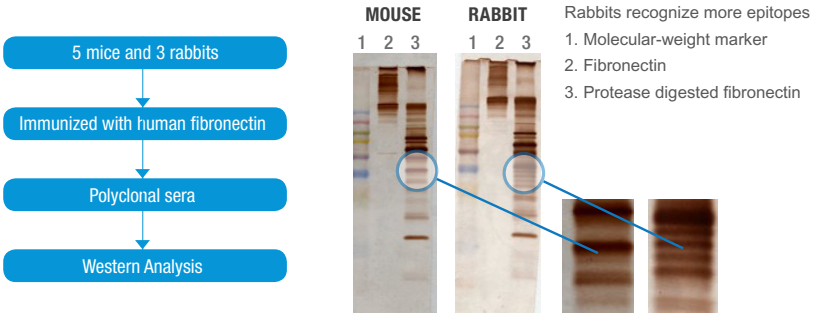


A comparison of Poly (ADP-ribose) polymerase (PARP-1) specific RabMAbs. Each PARP-1 RabMAb specifically recognizes specific cleavage site and with no cross-reactivity to non-specific cleavage sites.

6. Diverse/novel epitope recognition

The rabbit's immune system develops affinity in ways that are different than the mouse. These differences are largely attributed to the rabbit's lower immune dominance and larger B-cell repertoire. The benefit is a wider epitope recognition during antibody development. An example of this can be seen below, a comparison of rabbit and murine immune responses which shows that rabbit antisera recognizes a wider range of epitopes than mouse antibodies in a 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.

Immune response comparison: RabMAb vs. Mouse MAb



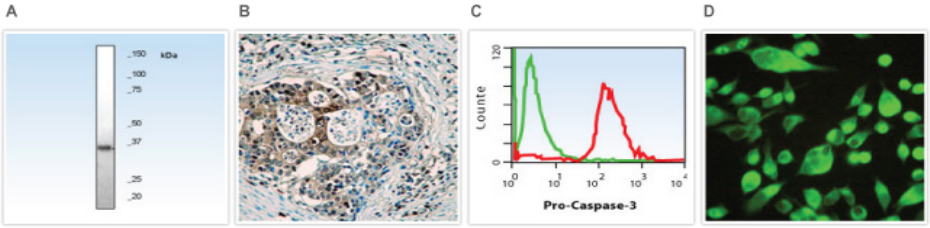
A comparison of rabbit and murine immune responses shows that rabbit antisera recognize more epitopes than mouse antibodies in a western blot analysis

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. By using a rabbit as a host with its unique mechanism of immune diversification and affinity maturation, RabMAbs can be produced to a wider range of epitopes, allowing discovery of more novel antibody targets.

7. Fully validated in multiple applications

Every RabMAb is tested in multiple applications (WB, IHC, ICC/IF, IP and FACS) and multiple species (Human, Mouse and Rat) before release. For IHC, all RabMAbs are tested on FFPE human tissue array for more accurate verification of antibody sensitivity and localization.

Validation results for Caspase-3 (Pro) RabMAb



Validation data for Caspase-3 (Pro) RabMAb (ab32150) on A) WB on HeLa cell lysate, B) IHC on FFPE human cervical carcinoma tissue, C) FACS on Jurkat cells and D) ICC on HeLa cells.

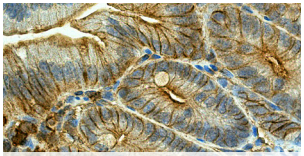
8. Ideal for use on mouse samples

RabMAbs are ideal reagents for customers using mouse models. 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, the RabMAb technology can produce a monoclonal antibody to mouse targets which are less immunogenic in mouse or rat. In addition, with RabMAbs, one can use monoclonal antibodies in mouse models without the issue of non-specific signal due to native mouse Ig cross-reactivity.

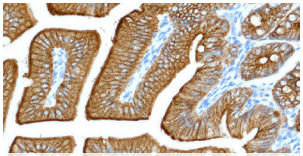
Every RabMAb has been tested for species cross-reactivity in western blotting using both human and mouse samples before release.

RabMAbs validated on mouse tissues by IHC



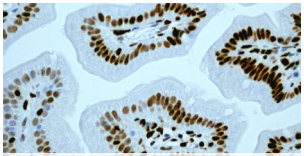
EGFR Phospho (pY1092)

IHC on FFPE mouse intestine using anti-EGFR (phospho Y1092) RabMAb (ab40815).



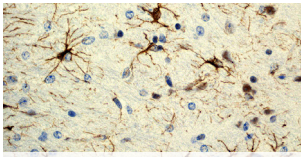
Cytokeratin 8

IHC on FFPE mouse colon using anti-Cytokeratin 8 RabMAb (ab53280).



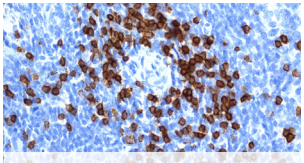
MCM2

IHC on FFPE mouse intestine using anti-MCM2 RabMAb (ab108935).



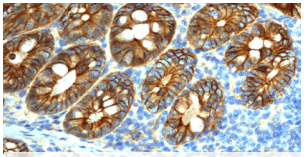
GFAP

IHC on FFPE mouse brain using anti-GFAP RabMAb (ab68428).



CD3 gamma

IHC on FFPE mouse spleen using anti-CD3 gamma RabMAb (ab134096).

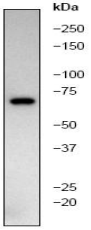


p120 Catenin

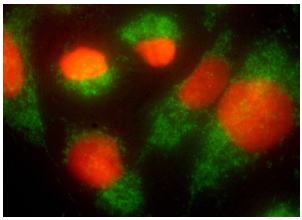
IHC on FFPE mouse large intestine using anti-p120 Catenin RabMAb (ab92514).

Featured RabMAbs

AIF antibody [E20] (ab32516)



WB using anti-AIF RabMAb on K562 cells.



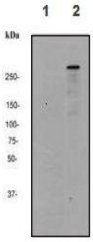
IF using anti-AIF RabMAb on HeLa cells.

Reactivity: Hu, Ms, Rt

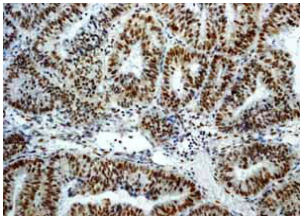
Applications: WB, IHC-F, IHC-P, ICC/IF, IP, FACS

Amount: 100 µl

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



WB using anti-ATM (phospho S1981) RabMAb antibody on HEK293 cell lysates. Lane 1: untreated, lane 2: doxorubicin treated.



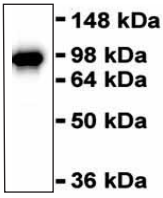
IHC using anti-ATM (phospho S1981) RabMAb on FFPE human endometrial carcinoma tissue.

Reactivity: Hu

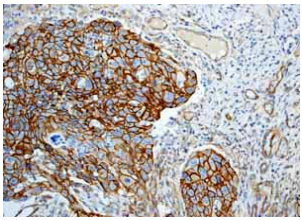
Applications: WB, IHC-P, ICC/IF, IP

Amount: 100 µl

beta Catenin antibody [E247] (ab32572)



WB using anti-beta Catenin RabMAb on whole cell lysate of U2OS cells.



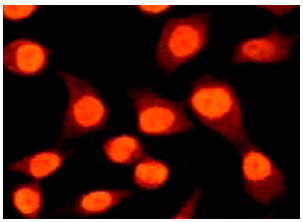
IHC using anti-beta Catenin RabMAb on FFPE human cervical carcinoma tissue.

Reactivity: Hu, Ms, Rt, Hm, Mk

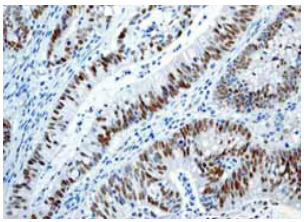
Applications: WB, IHC-F, IHC-P, ICC/IF, IP

Amount: 100 µl

c-Myc antibody [Y69] (ab32072)



IF using anti-c-Myc RabMAb on HeLa cells.



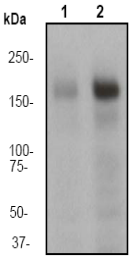
IHC using anti-c-Myc RabMAb on FFPE Human colonic adenocarcinoma tissue.

Reactivity: Hu, Ms, Rt

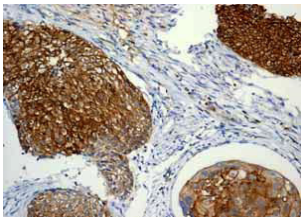
Applications: WB, IHC-P, ICC/IF, IP

Amount: 100 µl

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



WB using anti-EGFR (phospho Y1092) RabMAb on A431 cell lysates. Lane 1: EGF untreated, lane 2: EGF treated.



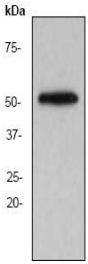
IHC using anti-EGFR (phospho Y1092) RabMAb on FFPE human cervical carcinoma tissue.

Reactivity: Hu, Ms

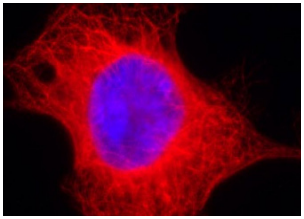
Applications: WB, IHC-F, IHC-P, ICC/IF

Amount: 100 µl

alpha Tubulin antibody [EP1332Y] (ab52866)



WB using anti-alpha Tubulin RabMAb on HeLa cell lysate at 10 µg.



IF using anti-alpha Tubulin RabMAb on human embryonic kidney cells.

Reactivity: Hu, Ms, Rt

Applications: WB, IHC-F, IHC-P, ICC/IF, IP, FACS

Amount: 100 µl

Discover more at abcam.com/RabMAbs