

### Anti-MyD88 antibody [EPR590(N)] ab133739

KO 評価済 リコンビナント RabMAb

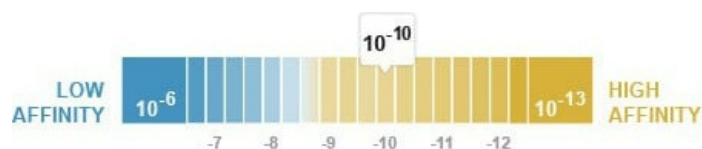
★★★★★ 1 Abreviews 38 References 画像数 13

#### 製品の概要

製品名	Anti-MyD88 antibody [EPR590(N)]
製品の詳細	Rabbit monoclonal [EPR590(N)] to MyD88
由来種	Rabbit
アプリケーション	適用あり: Flow Cyt (Intra), ICC/IF, WB, IHC-P
種交差性	交差種: Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Wild-type A549, HEK-293, Wild-type HAP1, Jurkat, MOLT4, K562, Raji, HepG2 and K562 cell lysates. IHC-P: Human cerebral cortex and kidney tissues. ICC/IF: Jurkat cells. Flow Cyt (intra): HAP1-WT, K562 and MCF7 cells.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Stable for 12 months at -20°C.
解離定数 (K <sub>D</sub> 値)	K <sub>D</sub> = 1.16 x 10 <sup>-10</sup> M



[Learn more about K<sub>D</sub>](#)

バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR590(N)
アイソタイプ	IgG

## アプリケーション

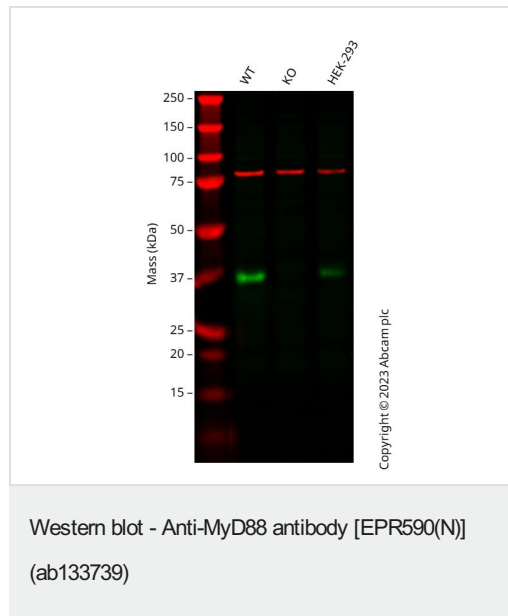
**The Abpromise guarantee**      **Abpromise保証は、次のテスト済みアプリケーションにおけるab133739の使用に適用されます**  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/350. For unpurified use 1/500 - 1/10000. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/500.
WB	★★★★★ (1)	1/1000 - 1/10000. Predicted molecular weight: 33 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <b><u>IHC antigen retrieval protocols</u></b> . <b>For unpurified, use 1/50 - 1/100.</b>

## ターゲット情報

機能	Adapter protein involved in the Toll-like receptor and IL-1 receptor signaling pathway in the innate immune response. Acts via IRAK1, IRAK2, IRF7 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. Increases IL-8 transcription. Involved in IL-18-mediated signaling pathway.
組織特異性	Ubiquitous.
関連疾患	Defects in MYD88 are the cause of MYD88 deficiency (MYD88D) [MIM:612260]; also known as recurrent pyogenic bacterial infections due to MYD88 deficiency. Patients suffer from autosomal recessive, life-threatening, often recurrent pyogenic bacterial infections, including invasive pneumococcal disease, and die between 1 and 11 months of age. Surviving patients are otherwise healthy, with normal resistance to other microbes, and their clinical status improved with age.
配列類似性	Contains 1 death domain. Contains 1 TIR domain.
ドメイン	The intermediate domain (ID) is required for the phosphorylation and activation of IRAK.

## 画像



**All lanes :** Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution

**Lane 1 :** Wild-type A549 cell lysate

**Lane 2 :** MYD88 knockout A549 cell lysate

**Lane 3 :** HEK-293 cell lysate

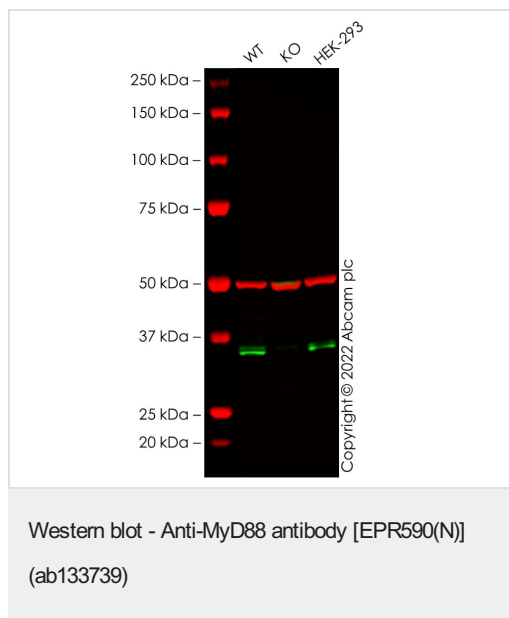
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 33 kDa

**Observed band size:** 35 kDa

Western blot: Anti-MYD88 antibody [EPR590(N)] (ab133739) staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] ([ab238078](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133739 was shown to bind specifically to MYD88. A band was observed at 35 kDa in wild-type A549 cell lysates with no signal observed at this size in MYD88 knockout cell line [ab286715](#) (knockout cell lysate [ab290793](#)). To generate this image, wild-type and MYD88 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



**All lanes :** Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution

**Lane 1 :** Wild-type A549 cell lysate

**Lane 2 :** MYD88 knockout A549 cell lysate

**Lane 3 :** HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

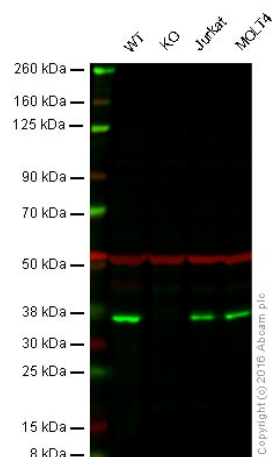
**All lanes :** Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

**Predicted band size:** 33 kDa

**Observed band size:** 35 kDa

False colour image of Western blot: Anti-MyD88 antibody [EPR590(N)] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] ([ab7291](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab133739 was shown to bind specifically to MyD88. A band was observed at 35 kDa in wild-type A549 cell lysates with no signal observed at this size in MYD88 knockout cell line [ab286715](#) (knockout cell lysate [ab290793](#)). To generate this image, wild-type and MYD88 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween<sup>®</sup> 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.



Western blot - Anti-MyD88 antibody [EPR590(N)]  
(ab133739)

**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

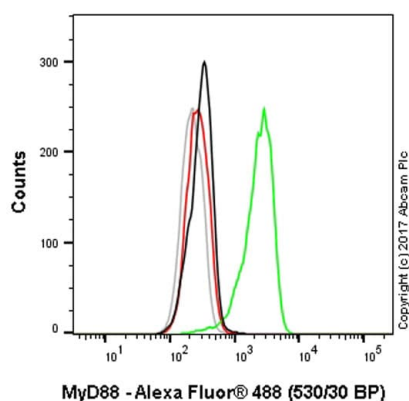
**Lane 2:** MyD88 knockout HAP1 cell lysate (20 µg)

**Lane 3:** Jurkat cell lysate (20 µg)

**Lane 4:** MOLT4 cell lysate (20 µg)

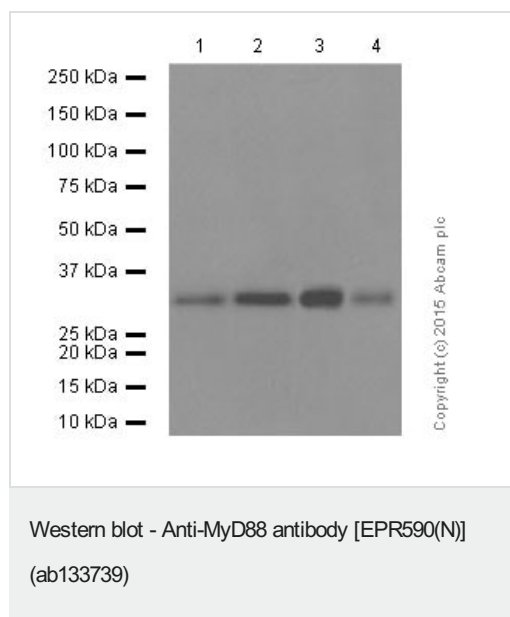
**Lanes 1 - 4:** Merged signal (red and green). Green - ab133739 observed at 37 kDa. Red - loading control, **ab7291**, observed at 52 kDa.

ab133739 was shown to specifically react with MyD88 when MyD88 knockout samples were used. Wild-type and MyD88 knockout samples were subjected to SDS-PAGE. ab133739 and **ab7291** (loading control to alpha tubulin) were diluted 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-MyD88 antibody  
[EPR590(N)] (ab133739)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-MyD88 knockout cells (red line) stained with ab133739. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab133739, 0.1 µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG1 isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MyD88 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



**All lanes :** Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/5000 dilution (purified)

**Lane 1 :** HepG2 cell lysate

**Lane 2 :** K562 cell lysate

**Lane 3 :** Raji cell lysate

**Lane 4 :** Jurkat cell lysate

Lysates/proteins at 29 µg per lane.

### Secondary

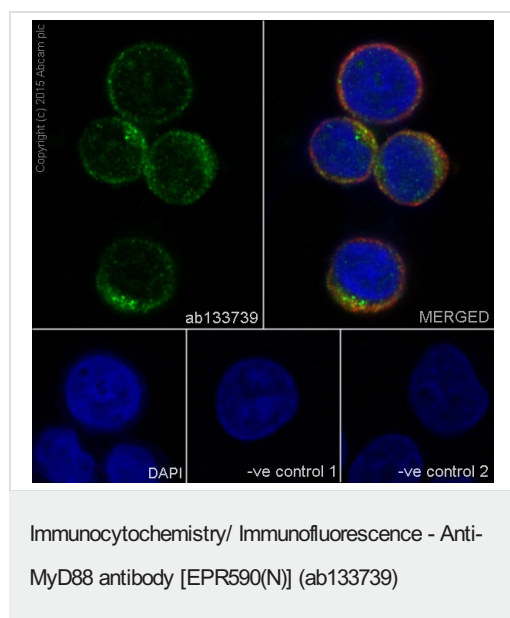
**All lanes :** HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

**Predicted band size:** 33 kDa

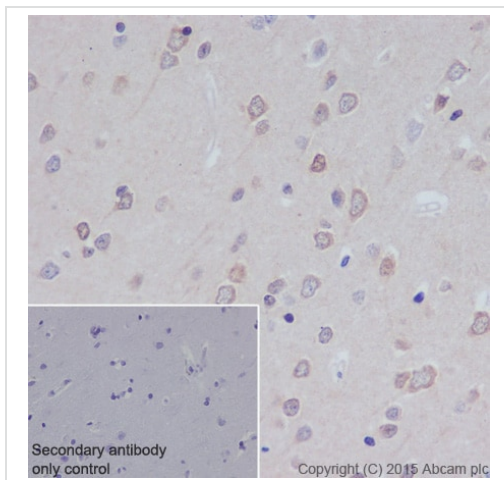
**Observed band size:** 33 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

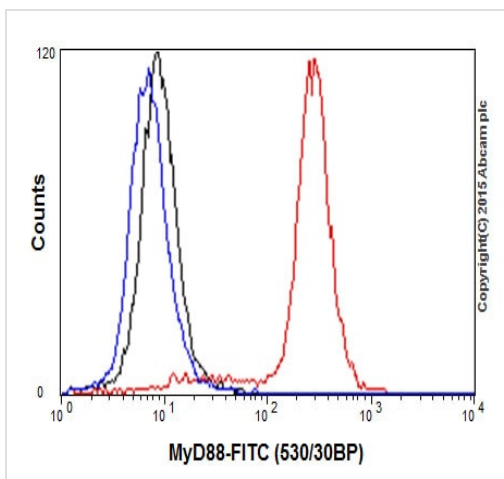


Immunofluorescence staining of Jurkat cells with purified ab133739 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit ([ab150077](#)), used at a dilution of 1/1000. [ab7291](#), a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with [ab150120](#) (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab133739 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody ([ab150120](#)) at a dilution of 1/500. For negative control 2, [ab7291](#) (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody ([ab150077](#)) at a dilution of 1/400.



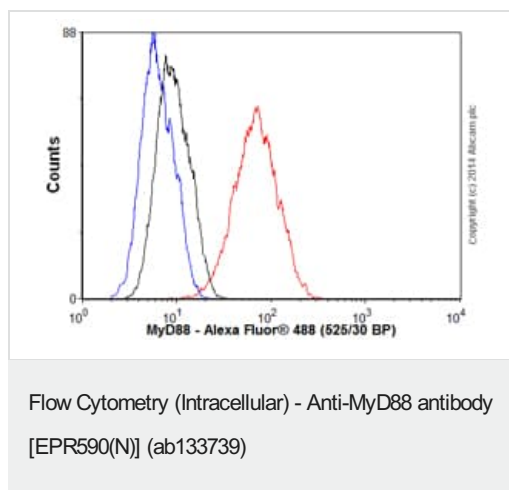
Immunohistochemical staining of paraffin embedded human cerebral cortex with purified ab133739 at a working dilution of 1/500. The secondary antibody used is HRP goat anti-rabbit IgG H&L ([ab97051](#)) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

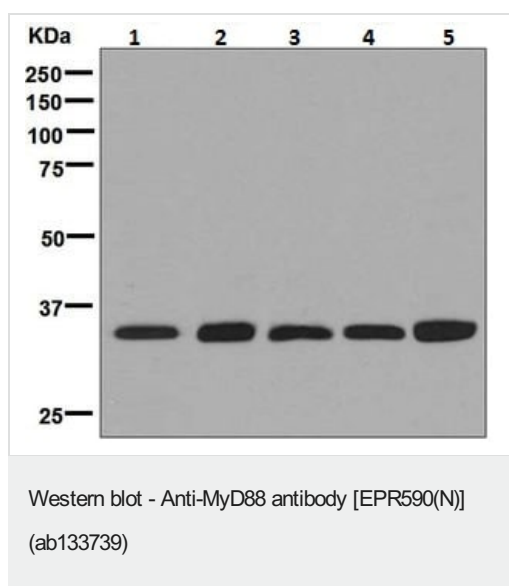


Overlay histogram showing K562 cells fixed in 4% PFA and stained with purified ab133739 at a dilution of 1 in 350 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

Flow Cytometry (Intracellular) - Anti-MyD88 antibody [EPR590(N)] (ab133739)



Overlay histogram showing MCF7 cells stained with unpurified ab133739 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab133739, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) ([ab150077](#)) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



**All lanes :** Anti-MyD88 antibody [EPR590(N)] (ab133739) at 1/1000 dilution (unpurified)

- Lane 1 :** Jurkat cell lysate
- Lane 2 :** Molt-4 cell lysate
- Lane 3 :** HepG2 cell lysate
- Lane 4 :** K562 cell lysate
- Lane 5 :** Raji cell lysate

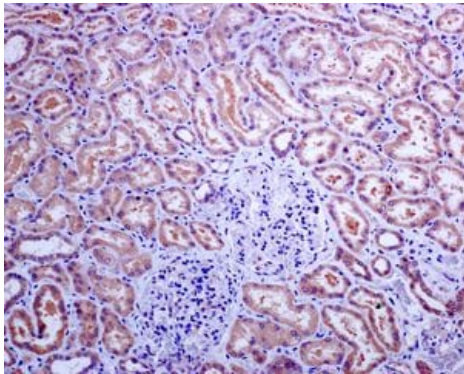
Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** HRP-conjugated goat anti-rabbit at 1/2000 dilution

**Predicted band size:** 33 kDa

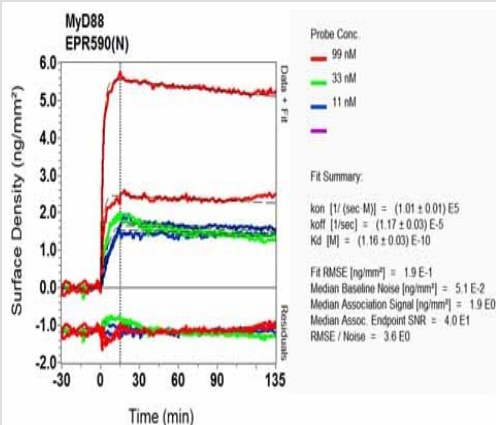




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MyD88 antibody [EPR590(N)] (ab133739)

Immunohistochemistry analysis of Myd88 expression in formalin-fixed, paraffin-embedded Human kidney tissue, using unpurified ab133739 at 1/50 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



SPR Scanning - Anti-MyD88 antibody [EPR590(N)] (ab133739)

Equilibrium dissociation constant ( $K_D$ )

Learn more about  $K_D$

[Click here to learn more about  \$K\_D\$](#)

Why choose a recombinant antibody?

 <p><b>Research with confidence</b> Consistent and reproducible results</p>	 <p><b>Long-term and scalable supply</b> Recombinant technology</p>
 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

Anti-MyD88 antibody [EPR590(N)] (ab133739)

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