

## Product datasheet

# Anti-Mark3 antibody ab77199

### 6 图像

#### 概述

<b>产品名称</b>	Anti-Mark3抗体
<b>描述</b>	小鼠单克隆抗体to Mark3
<b>宿主</b>	Mouse
<b>经测试应用</b>	<b>适用于:</b> WB, ICC/IF, Flow Cyt, ELISA
<b>种属反应性</b>	<b>与反应:</b> Human <b>预测可用于:</b> Mouse, Rat, Dog, Chimpanzee
<b>免疫原</b>	Recombinant fragment: HHKVQRSVFS SQKQRRYSDH AGPAIPSVVA YPKRSQTSTA DSDLKEDGIS SRKSSGSAVG GKGAPASPM LGNASNPNKA DIPERKKSST VPSSNTASG, corresponding to amino acids 402-500 of Human Mark3 (NP_002367) with a 26 kDa tag. <a href="#">Run BLAST with ExPASy</a> <a href="#">Run BLAST with NCBI</a>
<b>阳性对照</b>	Mark3 transfected 293T cell lysate. Recombinant tagged human Mark3 fragment.

#### 性能

<b>形式</b>	Liquid
<b>存放说明</b>	Shipped at 4°C. Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
<b>存储溶液</b>	Preservative: None Constituents: 1X PBS, pH 7.2
<b>纯度</b>	Protein A purified
<b>克隆</b>	单克隆
<b>同种型</b>	IgG2b
<b>轻链类型</b>	kappa

#### 应用

Our [Abpromise guarantee](#) covers the use of **ab77199** in the following tested applications.

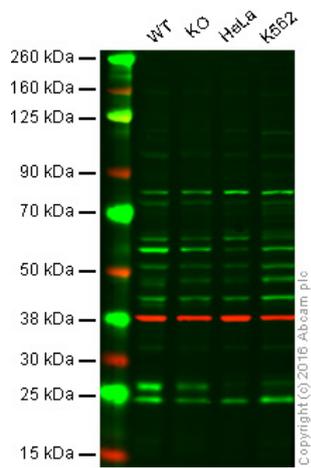
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

应用	Ab评论	说明
WB		Use a concentration of 1 - 5 µg/ml. Predicted molecular weight: 87 kDa.
ICC/IF		Use a concentration of 5 µg/ml.
Flow Cyt		Use 0.01-0.1µg for 10 <sup>6</sup> cells. <a href="#">ab170192</a> - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
ELISA		Use at an assay dependent concentration. Detection limit for recombinant tagged Mark3 is approximately 3ng/ml when used as a capture antibody.

## 靶标

功能	Involves in the specific phosphorylation of microtubule-associated proteins for tau, MAP2 and MAP4. Phosphorylates CDC25C on 'Ser-216'.
组织特异性	Ubiquitous.
序列相似性	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. MARK subfamily. Contains 1 KA1 (kinase-associated) domain. Contains 1 protein kinase domain. Contains 1 UBA domain.

## 图片



Western blot - Anti-Mark3 antibody (ab77199)

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

**Lane 2:** MARK3 knockout HAP1 cell lysate (20 µg)

**Lane 3:** HeLa cell lysate (20 µg)

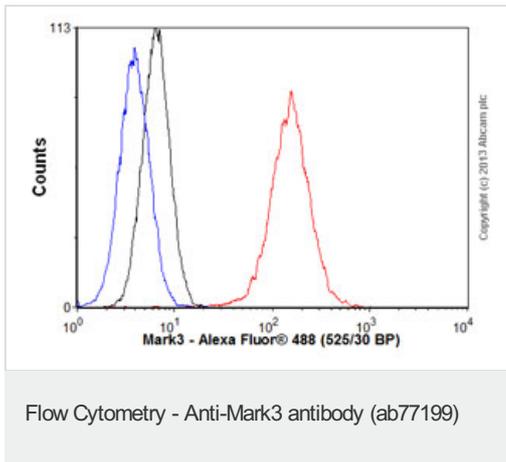
**Lane 4:** K562 cell lysate (20 µg)

**Lanes 1 - 4:** Merged (red and green) signal.

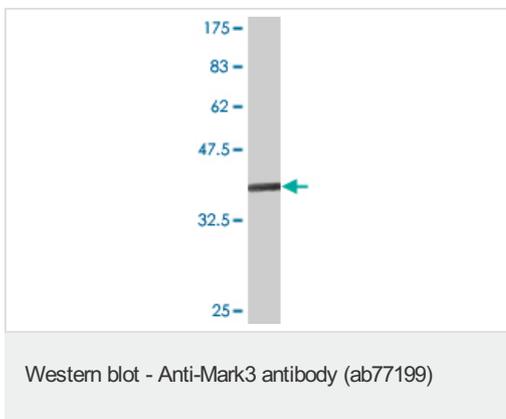
Green - ab77199 observed at 85 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab77199 was shown not to specifically react with MARK3, when MARK3 knockout samples were used. Wild-type and MARK3 knockout samples were subjected to SDS-PAGE.

ab77199 and ab181602 (loading control to GAPDH) were diluted at 1 µg/mL and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Overlay histogram showing HeLa cells stained with ab77199 (red line). The cells were fixed with 80% methanol (5 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 (ab77199, 0.01µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 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 HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Anti-Mark3 antibody (ab77199) at 5 µg/ml + Recombinant tagged human Mark3 fragment at 0.2 µg

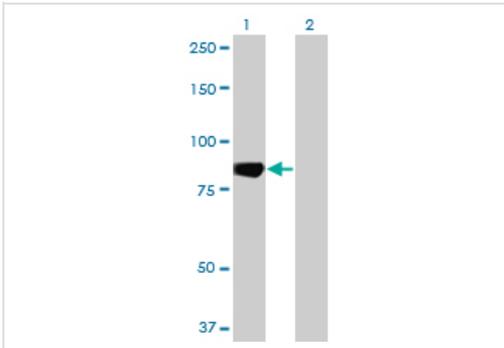
#### Secondary

Goat Anti-Mouse IgG (H&L)-HRP Conjugate at 1/5000 dilution

**Predicted band size:** 87 kDa

**Observed band size:** 37 kDa

Western blot against the tagged recombinant protein immunogen, which has a predicted molecular weight of 37 kDa.



Western blot - Anti-Mark3 antibody (ab77199)

**All lanes :** Anti-Mark3 antibody (ab77199) at 5 µg/ml

**Lane 1 :** Mark3 transfected 293T cell lysate

**Lane 2 :** Non transfected 293T cell lysate

Lysates/proteins at 25 µg per lane.

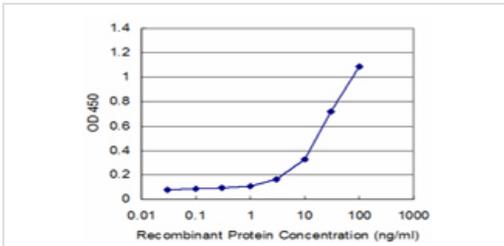
#### Secondary

**All lanes :** Goat Anti-Mouse IgG (H&L)-HRP

Conjugate at 1/2500 dilution

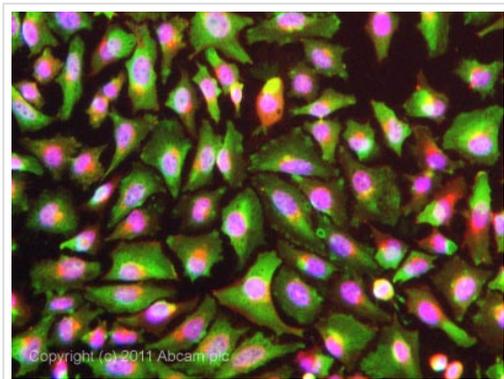
**Predicted band size:** 87 kDa

**Observed band size:** 87 kDa



ELISA - Anti-Mark3 antibody (ab77199)

Detection limit for ab77199 is approximately 3ng/ml as a capture antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Mark3 antibody (ab77199)

ICC/IF image of ab77199 stained HeLa cells.

The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab77199, 5µg/ml) overnight at +4°C. The secondary antibody (green) was [ab96879](#), DyLight® 488 goat anti-mouse IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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