# abcam

## Product datasheet

# Anti-Nck antibody [Y531] ab32120





重组 RabMAb

★★★★ 1 Abreviews 5 References 6 图像

概述

产品名称 Anti-Nck抗体[Y531]

描述 兔单克隆抗体[Y531] to Nck

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, Flow Cyt (Intra), ICC/IF

种属反应性 与反应: Mouse, Human

预测可用于: Rat 📤

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 Hela cell lysate and human breast carcinoma ICC/IF: HeLa cells

常规说明 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆 Y531 克隆编号 同种型 ΙgG

# The Abpromise guarantee Abpromise

Abpromise™承诺保证使用ab32120于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度:实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/2000 - 1/10000. Detects a band of approximately 47 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/1000.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/250.

靶	标

功能 Adapter protein which associates with tyrosine-phosphorylated growth factor receptors or their

cellular substrates. Maintains low levels of EIF2S1 phosphorylation by promoting its

dephosphorylation by PP1. Plays a role in the DNA damage response, not in the detection of the damage by ATM/ATR, but for efficient activation of downstream effectors, such as that of CHEK2.

序列相似性 Contains 1 SH2 domain.

Contains 3 SH3 domains.

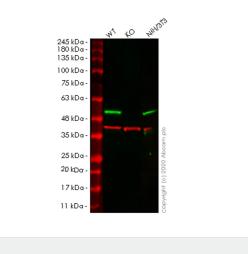
翻译后修饰 Phosphorylated on Ser and Tyr residues.

细胞定位 Cytoplasm. Endoplasmic reticulum. Nucleus. Mostly cytoplasmic, but shuttles between the

cytoplasm and the nucleus. Import into the nucleus requires the interaction with SOCS7. Predominantly nuclear following genotoxic stresses, such as UV irradiation, hydroxyurea or

mitomycin C treatments.

### 图片



Western blot - Anti-Nck antibody [Y531] (ab32120)

All lanes: Anti-Nck antibody [Y531] (ab32120) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: NCK1 knockout HeLa cell lysate

Lane 3: NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

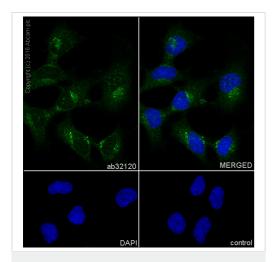
#### Secondary

**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Observed band size: 50 kDa

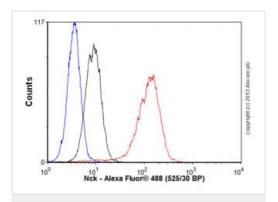
**Lanes 1-3:** Merged signal (red and green). Green - ab32120 observed at 50 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab32120 Anti-Nck antibody [Y531] was shown to specifically react with Nck in wild-type HeLa cells. Loss of signal was observed when knockout cell line <a href="mailto:ab265323">ab265323</a> (knockout cell lysate <a href="mailto:ab258059">ab258059</a>) was used. Wild-type and Nck knockout samples were subjected to SDS-PAGE. ab32120 and Anti-GAPDH antibody [6C5] - Loading Control (<a href="mailto:ab8245">ab8245</a>) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



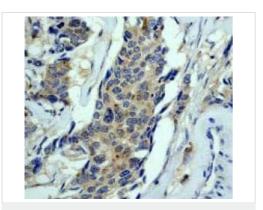
Immunocytochemistry/ Immunofluorescence - Anti-Nck antibody [Y531] (ab32120)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Nck with purified ab32120 at 1/250 dilution (8 µg/mL). Cells were fixed in 100% Methanol. Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain.



Flow Cytometry (Intracellular) - Anti-Nck antibody [Y531] (ab32120)

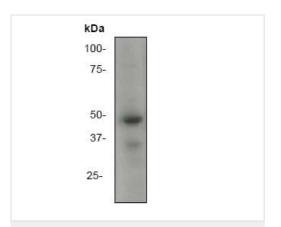
Overlay histogram showing Jurkat cells stained with ab32120 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32120, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor<sup>®</sup> 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 Jurkat cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Nck antibody [Y531] (ab32120)

ab32120 (1/100), staining human breast carcinoma by immunohistochemistry, Paraffin embedded tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



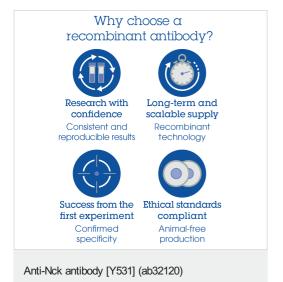
Western blot - Anti-Nck antibody [Y531] (ab32120)

Anti-Nck antibody [Y531] (ab32120) at 1/10000 dilution + Hela cell lysate

Observed band size: 47 kDa

Additional bands at: 35 kDa. We are unsure as to the identity of

these extra bands.



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