

DESCRIPTION

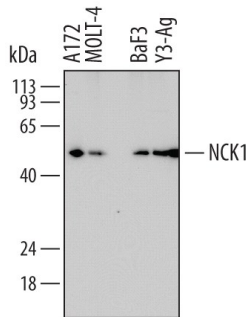
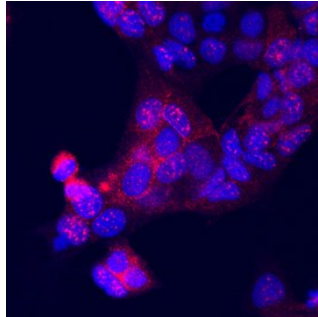
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|---------------------------|---|
| Species Reactivity | Human |
| Specificity | Detects human NCK1 in direct ELISAs. |
| Source | Monoclonal Mouse IgG _{2A} Clone # 714506 |
| Purification | Protein A or G purified from hybridoma culture supernatant |
| Immunogen | <i>E. coli</i> -derived recombinant human NCK1 Met1-Ser377 Accession # P16333 |
| Formulation | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS. |

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

| | Recommended Concentration | Sample |
|----------------------------|----------------------------------|---------------|
| Western Blot | 1 µg/mL | See Below |
| Immunocytochemistry | 8-25 µg/mL | See Below |

DATA

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| <p>Western Blot</p>  | <p>Detection of Human, Mouse, and Rat NCK1 by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line, MOLT-4 human acute lymphoblastic leukemia cell line, BaF3 mouse pro-B cell line, and Y3-Ag rat myeloid cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human NCK1 Monoclonal Antibody (Catalog # MAB7008) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for NCK1 at approximately 47 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p> | <p>Immunocytochemistry</p>  <p>NCK1 in MCF-7 Human Cell Line. NCK1 was detected in immersion fixed MCF-7 human breast cancer cell line using Mouse Anti-Human NCK1 Monoclonal Antibody (Catalog # MAB7008) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p> |
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PREPARATION AND STORAGE

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| Reconstitution | Sterile PBS to a final concentration of 0.5 mg/mL. |
| Shipping | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C |
| Stability & Storage | Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution. |

BACKGROUND

NCK1 (Non-catalytic region of tyrosine kinase 1; also Sh2/SH3 adaptor protein NCK-α) is a 46-48 kDa member of the Nck family of adaptor proteins. NCK1 is ubiquitously expressed and may be found in both cytoplasm and nucleus. In the cytoplasm, it connects tyrosine kinases to actin reorganization which, in T cells, involves SLP-76 and VAV1. Upon DNA damage, NCK1 binds SOCS7 and is translocated into the nucleus where it participates in p53 phosphorylation and cell cycle arrest. Human NCK1 is 377 amino acids (aa) in length. It contains three N-terminal SH3 domains (aa 2-252) that mediate binding to Pro-rich regions of cytoplasmic proteins, and a C-terminal SH2 domain (aa 282-376) that binds to phosphorylated proteins or receptors. There are at least three utilized Ser, and one utilized Tyr phosphorylation sites. There are also three potential splice variants. One shows a 12 aa substitution for aa 1-76, a second possesses an 11 aa substitution for aa 77-377, and a third contains a 37 aa substitution for aa 134-377. Full-length NCK1 shares 99% aa identity with mouse NCK1.

MAB7008 detects mouse and rat NCK1 in Western Blots. Cross-reactivity with mouse and rat samples in immunocytochemistry has not been tested.