Human Cyclin E1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6810

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Cyclin E1 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Cyclin E1 Met16-Lys143 Accession # P24864
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.

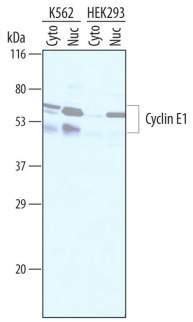
APPLICATIONS

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
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	50 µg/mL	See Below

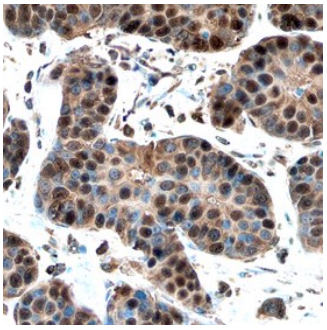
DATA

Western Blot



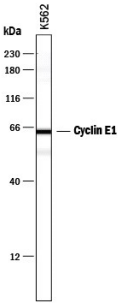
Detection of Human Cyclin E1 by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line and HEK293 human embryonic kidney cell line. Gels were loaded with 25 µg of cytoplasmic (Cyto) and 25 µg of nuclear (Nuc) extracts. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Cyclin E1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6810) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for Cyclin E1 at approximately 50 - 60 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunohistochemistry



Cyclin E1 in Human Breast. Cyclin E1 was detected in immersion fixed paraffin-embedded sections of human breast using Sheep Anti-Human Cyclin E1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6810) at 10 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human Cyclin E1 by Simple Western™. Simple Western lane view shows lysates of K562 human chronic myelogenous leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Cyclin E1 at approximately 64 kDa (as indicated) using 50 µg/mL of Sheep Anti-Human Cyclin E1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6810) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 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.** ● 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.

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BACKGROUND

Cyclin E1 (also known as G1/S-specific cyclin-E1 and CCNE1) is a 48-58 kDa member of the cyclin E subfamily, cyclin family of molecules. It associates with Cdk2 kinase, and determines substrate specificity for the complex. This complex phosphorylates multiple substrates involved in cell cycle progression and the initiation of DNA replication. Cyclin E1 is required for G1/S phase progression and cell cycle reentry from G0 phase. Its reduced activity during cellular senescence contributes to G1 arrest. Human cyclin E1 is 410 amino acids (aa) in length. It contains two cyclin box folds (aa 144-234 and 293-363) and is phosphorylated on at least eight Ser/Thr sites. There are at least two alternate splice forms. One is 40-41 kDa in size and utilizes an alternate start site at Met46, while a second is 43-44 kDa in size and shows a deletion of aa 154-196. One other potential start site exists at Met16. There are multiple proteolytic cleavage products that are tumor-associated and show increased destabilizing activity. Cleavage around Gln40 generates 44-45 kDa C-terminal fragments, while cleavage between Ala69Asp70 generates 33-35 kDa C-terminal fragments. Over aa 16-143, human cyclin E1 shares 67% aa identity with mouse cyclin E1.

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