

DESCRIPTION

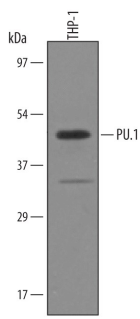
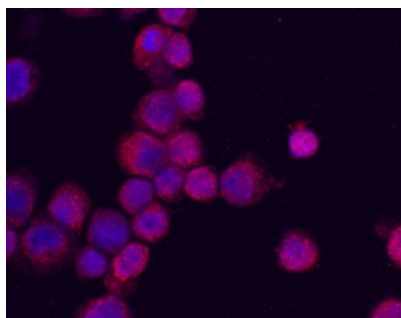
Species Reactivity	Human
Specificity	Detects human PU.1 in direct ELISAs and Western blots. In direct ELISAs, approximately 5% cross-reactivity with recombinant human SPI-B is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human PU.1 Met1-Lys169 Accession # NP_001074016
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	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10 ⁶ cells	PMA and Ca ²⁺ ionomycin-treated human Th2-stimulated PBMCs, fixed with paraformaldehyde, and permeabilized with saponin

DATA

<p>Western Blot</p>  <p>Detection of Human PU.1 by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line. PVDF membrane was probed with 1 µg/mL of Human PU.1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5870) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for PU.1 at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.</p>	<p>Immunocytochemistry</p>  <p>PU.1 in THP-1 Human Cell Line. PU.1 was detected in immersion fixed THP-1 human acute monocytic leukemia cell line using Human PU.1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5870) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to nuclei and cytoplasm. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
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

PU.1 (Purine-rich nucleic acid binding protein 1; also 31 kDa transforming protein and SPI-1) is a member of the PU subfamily, ETS family of transcription factors. Although its predicted MW is 31 kDa, it appears to run anomalously high in SDS-PAGE at 40-45 kDa. PU.1 is a monomeric hematopoietic protein that regulates the differentiation of early myeloid and lymphoid progenitors. High PU.1 levels favor granulocyte and macrophage production, while low levels generate megakaryocytes, erythrocytes, T and B cells. Human PU.1 is 270 amino acids (aa) in length. It contains an N-terminal acidic/polyGln transactivation region (aa 34-99), a non-destabilizing PEST sequence (aa 117-165) and a C-terminal Ets DNA-binding domain (aa 170-253). PU.1 is phosphorylated on Ser146, allowing it to bind to Pip. Over aa 1-169, human PU.1 shares 88% aa identity with mouse PU.1.