

**#2266** Store at -20°C

# PU.1 Antibody



100 µl  
 (10 western blots)

**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignal.com  
**Web** ■ www.cellsignal.com

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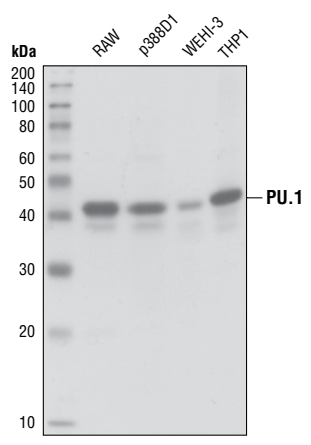
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, ChIP, IF-IC, F Endogenous	H, M, (Mk, Pg)	42 kDa	Rabbit**

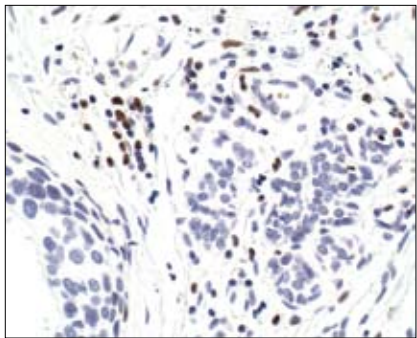
**Background:** PU.1 is a member of the Ets family of transcription factors and activates target genes through the purine-rich PU-box (1). PU.1 plays a pivotal role in the differentiation of myeloid cells and lymphocytes and is expressed in several hematopoietic cells including B lymphocytes, macrophages, neutrophils, mast cells, early erythroid cells and megakaryocytes (1,2). The concentration of PU.1 is critical for both the determination of hematopoietic cell lineage and the regulation of differentiation versus stem cell proliferation (3,4). In addition, PU.1 activity is influenced by phosphorylation and interactions with other hematopoietic transcription factors. Phosphorylation of PU.1 at Ser146 by CK2 promotes binding to IRF4 and synergistic activation through the immunoglobulin κ 3' enhancer (5). Treatment of pro-B cells with IL-3 leads to phosphorylation of PU.1 at Ser140, resulting in increased PU.1 activity and activation of the anti-apoptotic gene MCL-1 (6). GATA1 binding blocks PU.1 activity during erythroid cell development (7). Overexpression of PU.1 resulting from proviral insertion during Friend virus infection can induce erythroleukemia, while reduced expression has been associated with acute myeloid leukemia (8).

**Specificity/Sensitivity:** This antibody detects endogenous levels of total PU.1 protein. The antibody does not cross react with other Ets family members.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino acids at the amino-terminus of human PU.1. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from RAW, p388D1, WEHI-3 and THP1 cells using #2266.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing nuclear localization in lymphocytes, using PU.1 Antibody.

**Entrez-Gene ID #** 6688  
**Swiss-Prot Acc. #** P17947

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by western blot.**

**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**

Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:200
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS
Immunofluorescence (IF-IC)	1:100
Chromatin IP	1:25
Flow Cytometry	1:50

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

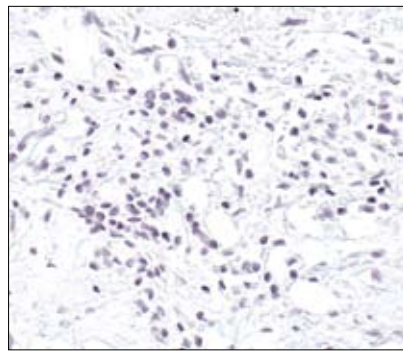
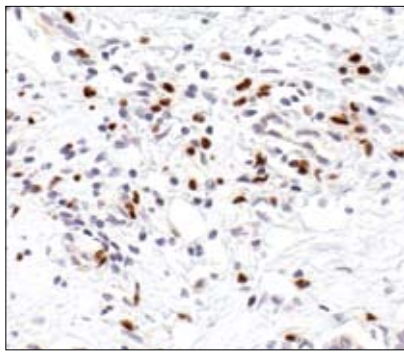
Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Background References:**

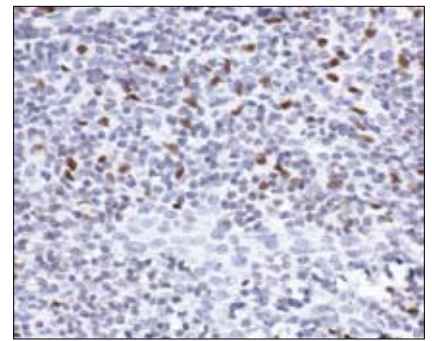
- (1) Lloberas, J. et al. (1999) *Immunol. Today* 20, 184–189.
- (2) Klemsz, M.J. et al. (1990) *Cell* 61, 113–124.
- (3) Dahl, R. and Simon, M.C. (2003) *Blood Cells Mol. Dis.* 31, 229–233.
- (4) DeKoter, R.P. and Singh, H. (2000) *Science* 288, 1439–1441.
- (5) Pongubala, J.M. et al. (1993) *Science* 259, 1622–1625.
- (6) Wang, J.M. et al. (2003) *Mol. Cell Biol.* 23, 1896–1909.
- (7) Zhang, P. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 8705–8710.
- (8) Moreau-Gachelin, F. et al. (1998) *Nature* 331, 277–280.

**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

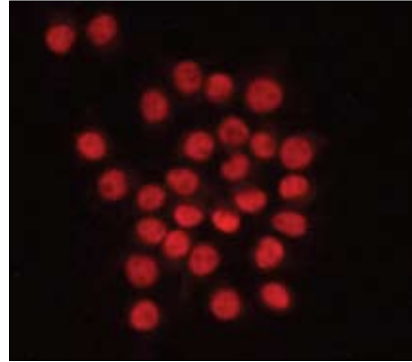
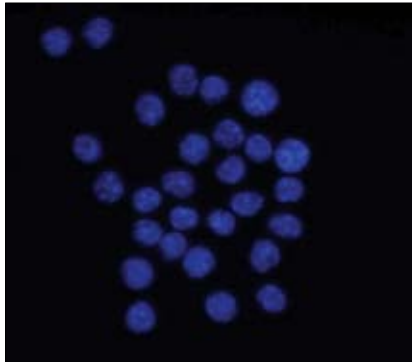
**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



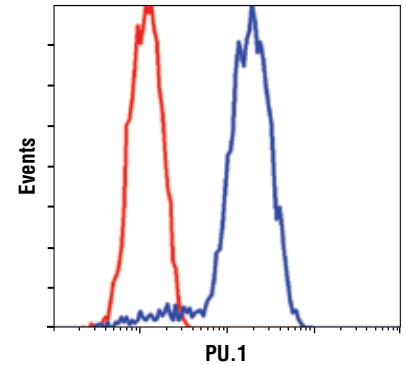
Immunohistochemical analysis of paraffin-embedded human breast carcinoma (infiltrating cells), using PU.1 Antibody in the presence of control peptide (left) or PU.1 Blocking Peptide #1036 (right).



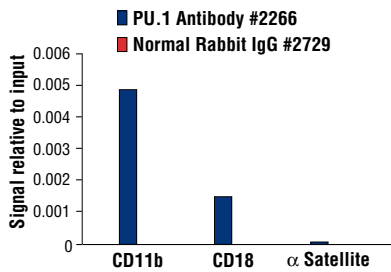
Immunohistochemical analysis of paraffin-embedded human tonsil, showing nuclear localization, using PU.1 Antibody.



DAPI staining (left) and immunofluorescent staining (right) of paraformaldehyde-fixed RAW cells using #2266.



Flow cytometric analysis of RAW cells, using PU.1 antibody (blue) compared to a nonspecific negative control antibody (red).



Chromatin immunoprecipitations were performed with cross-linked chromatin from  $4 \times 10^6$  U-937 cells and either 20  $\mu$ l of PU.1 Antibody or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using human CD11b promoter primers, human CD18 intron 1 primers, and SimpleChIP™ Human  $\alpha$  Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.