Human WT1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF5729

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human WT1 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human WT1 Met127-Gly249 Accession # P19544
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.

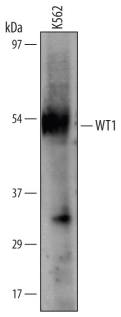
APPLICATIONS

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
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

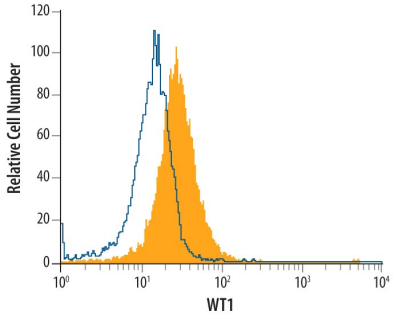
DATA

Western Blot



Detection of Human WT1 by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human WT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5729) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for WT1 at approximately 54 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Intracellular Staining by Flow Cytometry



Detection of WT1 in HL-60 Human Cell Line by Flow Cytometry. HL-60 human acute promyelocytic leukemia cell line was stained with Goat Anti-Human WT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5729, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

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.** ● 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

WT1 (Wilms' tumor protein 1; also WT33) is a 52-54 kDa, nuclear member of the EGR C2H2-type zinc-finger family of proteins. Although its predicted MW is 49 kDa, it runs anomalously in SDS-PAGE, likely due to a high proline content. It is widely expressed, being found in developing Sertoli cells, glomerular podocytes, neurons, and CD34+ stem cells. Human WT1 is 449 amino acids (aa) in length. It contains a Pro-rich domain (aa 27-83) and four consecutive C2H2 zinc finger regions (aa 323-347; 353-377; 383-405; 414-438). WT1 forms homodimers, and interacts with multiple molecules. Interaction with the zinc fingers generally promotes gene transcription, while N-terminal interactions block gene transcription. There are at least two dozen splice variants. Some are combinations of deletions of aa 250-266 and 408-410, plus an alternate start site 68 aa upstream of the standard site, and a three aa substitution for aa 1-147. Over aa 127-249, human WT1 shares 98% aa identity with mouse WT1.

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