

## ORDERING INFORMATION

**Catalog Number:** AF5729

**Lot Number:** CBTN01

**Size:** 100 µg

**Formulation:** 0.2 µm filtered solution in PBS with 5% trehalose

**Storage:** -20° C

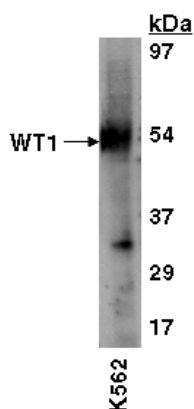
**Reconstitution:** sterile PBS

**Specificity:** human WT1

**Immunogen:** *E. coli*-derived rhWT1 (aa 127 - 249)

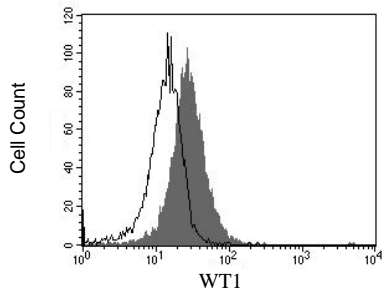
**Ig Type:** goat IgG

**Applications:** Western blot  
Flow cytometry  
Direct ELISA



### Detection of WT1 with AF5729.

Cell lysates were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL goat anti-hWT1.



HL-60 cells were stained with anti-WT1 (R&D Systems, Cat # AF5729, filled histogram), or control antibody (R&D Systems, Cat. # AB-108-C, open histogram) followed by APC-conjugated anti-goat IgG (R&D Systems, Cat. # F0108).

## 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.

## Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human WT1 (rhWT1; aa 127 - 249; Accession # P19544). Human WT1 specific IgG was purified by human WT1 affinity chromatography.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute with sterile PBS. If 0.5 mL of PBS is used, the antibody concentration will be 0.2 mg/mL.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a **manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

## Specificity

This antibody has been selected for its ability to recognize human WT1 in the applications listed below.

## Applications

**Western blot** - An antibody concentration of 1.0 µg/mL is recommended.

**Flow cytometry** - This antibody was tested for flow cytometry using HL-60 cells. For intracellular staining to detect WT1, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 x 10<sup>5</sup> cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-goat IgG conjugated to a fluorochrome.

**Direct ELISA** - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human WT1. The detection limit for rhWT1 is approximately 1 ng/well.

**Optimal dilutions should be determined by each laboratory for each application.**