MONOCLONAL MOUSE
ANTI-Human Wilms’ Tumor 1 (WT1) Protein, Clone 6F-H2
CODE NO. M3561
LOT NO. 051

For In Vitro Diagnostic Use

Immunogen
Truncated human WT1 protein corresponding to N-terminal amino acids 1-181.1,2

Clone
6F-H2.1,2

Presentation
Anti-human WT1, 6F-H2 is a mouse monoclonal antibody supplied in liquid form as tissue culture supernatant (containing fetal bovine serum) dialyzed against 0.05mol/L Tris-Cl, pH 7.2, and 15mmol/L sodium azide.

Total Protein Concentration: 13.9 mg/mL (Refractometry)
Mouse IgG Concentration: 630 µg/mL (Single Radial Immunodiffusion)
Subclass: IgG1, kappa

Specificity
WT1 is a gene involved in the induction of Wilms’ tumor, a pediatric renal malignancy. The WT1 gene, located on chromosome 11p13, is inactivated in 5 to 10% of sporadic Wilms’ tumors and nearly 100% of Denys-Drash patients, a syndrome associated with genitourinary abnormalities and Wilms’ tumor. WT1 encodes a zinc finger transcription factor which recognizes the early growth response (EGR-1) consensus sequence found in promoters of growth factor genes. The protein encoded by WT1 regulates transcription of other genes and can function both as a transcriptional activator and repressor. WT1 has been demonstrated to repress transcription of various growth-related genes such as platelet-derived growth factor A chain (PDGF-A) and insulin-like growth factor (IGF).3-5 The transcriptional activity of this protein, however, can be modulated through physical interactions between WT1 and p53. In the absence of wild type p53, WT1 has been demonstrated to function as a transcriptional activator of the EGR-1 promoter in transfection assays.6

Alternate splicing of the WT1 transcript gene results in four distinct mRNA species which are present in different amounts. The predicted molecular weight of the WT1 protein ranges from 46 to 49 kD depending on the mRNA species however, the WT1 proteins derived from cells migrate at 50 to 55 kDa in SDS-PAGE.4 Monoclonal mouse anti-human WT1 (anti-WT1) recognizes an epitope found in the amino terminal 84 amino acids of WT1. Anti-WT1 reacts with all isoforms of the full-length WT1 and also identifies WT1 lacking exon 2, frequently found in subsets of sporadic Wilms’ tumors. In immunoprecipitation assays, anti-WT1 has been shown to recognize full-length and in vitro translated WT1. Anti-WT1 also detects full-length denatured WT1 (55 kDa) in Western blots of WT1 baculovirus-infected cell lysates.1

Reactivity
Normal cells
WT1 mRNA is strongly expressed in mesenchymally derived tissue. During embryonic development, WT1 expression has been observed in human kidney, spleen, and gonadal ridge mesoderm and the mesothelial lining of the coelomic cavity and the organs contained within.7 WT1 gene expression in adult human tissues has been observed in sertoli cells of testes, decidual cells of the uterus and in granulosa cells of the ovary. A number of other adult tissues were shown to contain WT1 transcripts including pleura, spleen, heart, foreskin, endometrium, and glomerular epithelium of the kidney.8 Antibodies to WT1, including clone 6F-H2, have been demonstrated to stain the cytoplasm of the following normal cells in some tissue specimens. Cytoplasmic staining of blood vessels and connective tissue of lung alveoli were observed to stain positively.9 Granular cytoplasmic staining has also been reported in mature myelocytic cells.10
Tumor cells
High levels of WT1 expression have been demonstrated in the epithelial and blastemal components of Wilms' tumors, whereas stromal elements were found to be expressed at very low levels. The majority of malignant mesotheliomas were found to be strongly express WT1 message and also the gene product. The WT1 protein was immunolocalized, using anti-WT1 (clone 6F-H2), to the nuclei of malignant mesothelioma cells. WT1 gene and protein expression have also been demonstrated in the majority of acute leukemias but not in cells from chronic myelogenous leukemia, except when in blast crisis. Anti-WT1 immunostained the nuclei of leukemia blast cells but was unreactive with normal mononuclear cells and normal peripheral CD34+ hematopoietic progenitor cells. WT1 mRNA expression has been reported in a number of other tumors including melanomas, ovarian cancer and sex cord-stromal tumors. In addition to specific nuclear immunoreactivity, antibodies to WT1 (including clone 6F-H2) have been reported to stain the cytoplasm of tumor cells in some cases of adenocarcinoma and the desmoplastic stroma and basement membrane of some carcinoma specimens. Perinuclear staining of tumor cells in malignant mesothelioma has also been observed. This cytoplasmic staining may represent cross-reactivity with an epitope unrelated to WT1.

Staining Procedure
Paraffin Sections
Anti-human WT1 can be used on formalin-fixed, paraffin-embedded tissue sections. Tissue sections should be pretreated with a 0.4% pepsin solution in 0.2N HCl for 30 minutes at 37°C. For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO Code No. S3003) is recommended.

Anti-human WT1 may be used at a dilution of 1:50 in the LSAB+ method determined on formalin-fixed, paraffin-embedded tissue. These are guidelines only; optimal dilutions should be determined by the individual laboratory.

Cryostat Sections and Cell Smears
Anti-human WT1 can also be use to label cryostat sections or cell smears.

Storage
Store at 2-8°C or -20°C. Avoid repeated freeze-thaw cycles.

References