MONOCLONAL MOUSE
ANTI-METALLOTHIONEIN (MT)
(DAKO-MT, E9)
CODE NO. M0639
LOT NO. 050

Immunogen
Horse self-polymerized MT-1 and MT-2

Clone/Ref
E9

Presentation
DAKO-MT, E9 is a monoclonal mouse antibody from ascites supplied in liquid form in 0.05M Tris-HCl, 15mM NaN3, pH 7.5, 1% BSA.
Protein Concentration: 1.1 mg/mL (Refractometry, excluding carrier protein)
Mouse IgG Concentration: 115 µg/mL (Single Radial Immunodiffusion)
Subclass: IgG1, kappa

Specificity
The human genome contains two separate groups of genes, MT-1 and MT-2, comprising twelve distinct MT genes, of which only six or seven encode functional proteins.1-3 Protein synthesis may be increased by many factors including divalent heavy metals, hormones, growth factors, cytokines, tumor promoters, UV-damaged DNA and others.2-9 In man, the induction of only the MT-1 protein is metallospecific.10

The products these genes encode are a group of low molecular weight proteins (MW ≈ 6 kD) containing a single chain of 61 amino acids which is folded doubly within two domains, A and B.1 The dominant and apparently only immunoreactive site resides within the last five to seven amino acids of the N-terminus of the B domain. The molecules contain 20 reduced cysteine residues which can chelate seven bivalent heavy metal ions through mercaptide bonds.7,9

DAKO-MT, E9 is inhibited specifically and equally well by glutaraldehyde-polymerized human, horse, sheep or rat MT-1 and MT-2, suggesting that it is directed against a single and highly conserved epitope.

Reactivity
Immunohistochemical (IHC) localization of MT has shown its rather ubiquitous presence in both cytoplasm and nuclei of many normal cell types.8,12 Strong expression of MT was noted in many epithelial structures, especially simple epithelia and basal cells, myoepithelia and trabecular epithelia. Also stained were peripheral nerves and smooth muscle cells.12 Parenchyma of lung, liver and pancreas stained focally.12

In the normal developing human liver, DAKO-MT, E9 stains cytoplasmic and nuclear elements in fetuses and children up to approximately six years of age. In the adult liver, staining is weaker and diffusely cytoplasmic.11,13 Polymerized lysosomal MT, responsible for 75% of tissue copper binding, does not react with DAKO-MT, E9.13 In cadmium over-exposure and in Wilson's disease hepatic staining for MT is intensified and appears "clustered", whereas decreased hepatic staining was found in cirrhosis, hepatitis and in malignancies.1
Normal breast showed strong immunoreactivity for MT in myoepithelia of ducts and only weak focal staining of lobuli.\textsuperscript{12,14,15} Of 21 patients with ductal breast carcinomas whose tumors were tested for MT expression, 10 patients with strong immunoreactivity had shorter survival time than did the remainder of patients whose tumors either unstained or only weakly positive.\textsuperscript{14} A somewhat poorer prognosis was found in patients who were estrogen receptor negative and MT positive. In seven of nine patients with invasive lobular carcinoma and weakly positive MT staining, five showed an unfavorable course.\textsuperscript{14}
44% of breast carcinomas and in most cases was either nuclear only or both nuclear and cytoplasmic. An inverse relationship was found between MT immuno-taining and estrogen receptor content of tumor and a statistically significant association was shown between MT immunostaining and histologic grade (P <0.01) as well as nuclear grade (P <0.01) of breast carcinoma. All positive tumors were invasive ductal carcinomas, including one medullary and one metablastic carcinoma. Mucinous, lobular or intraductal papillary carcinomas showed no reactivity for MT.15

IHC localization of MT in thyroid tissue showed two of ten normal glands to express MT and 91% of 34 thyroid tumors to be positive.16 The three different staining patterns observed, i.e. nuclear only, cytoplasmic only and nuclear as well as cytoplasmic, could not be explained by the type of tumor.

Staining Procedure

**PARAFFIN SECTIONS**

DAKO-MT, E9 can be used on formalin-fixed paraffin-embedded tissue sections.

A variety of staining techniques is suitable, including avidin-biotin methods, the APAAP (alkaline phosphatase anti-alkaline phosphatase) and PAP (peroxidase anti-peroxidase) techniques.

DAKO-MT, E9 may be used at a dilution of 1:50 in LSAB methods 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**

DAKO-MT, E9 can also be used to label cryostat sections or cell smears.

Storage

Store at 2-8°C or below 0°C. Avoid repeated freeze-thaw.

References

12. DAKO Corporation, unpublished observations