ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Tyrosine Protein Kinase Receptor (TIE-2) Antibodies

Cat. TIE22-S	Rabbit Anti-Human Tie-2 Antiserum # 2	**SIZE**: 100 ul
Cat. TIE22-A	Rabbit Anti-Human Tie-2 IgG # 2(aff pure)	**SIZE**: 100 ug
Cat. TIE22-P	Human Tie-2 Control/blocking peptide # 2	**SIZE**: 100 ug
Cat. TIE22-C	Recombinant Human Tie-2-Fc protein for WB	**SIZE**: 100 ul

Embryonic vascular system undergoes a series of complex, highly regulated series of events involving differentiation, migration and association of primitive endothelial cells. This process is termed vasculogenesis. A further remodeling of the primitive vascular system forms the mature cardiovascular system. This process is known as angiogenesis (sprouting of new capillary vessels from pre-existing vasculature). A family of receptor tyrosine kinases **TIE1 and TIE 2** or Tek has been identified in vascular endothelium and hematopoietic cells. Mice lacking TIE 1 or TIE 2 are lethal. Ties may represent the earliest endothelial cell lineage marker and may regulate the endothelial cell proliferation, differentiation, and proper patterning during vasculogenesis. TIEs appear to be acting downstream of the VEGFRs. **Tie-2** (human 1124 AA; mouse 1122 aa) is a type 1 membrane receptor protein specifically expressed in developing vascular endothelial cells and their progenitors, angioblasts. It is also found in placenta and lung, with lower levels in umbilical vein endothelial cells, brain and kidney. Tie-2 extracellular portion (25-279 aa) contains 3 fibronectin type III-like and 2-Ig-like C2-type, and 3-EGF-like domains.

Source of Antigen and Antibodies

Antigen	18-aa peptide of Human TIE2 ; **Designated (TIE22-P or control peptide)** conjugated to KLH
Location	~N-terminal, Extracellular
Ab Host/type	Rabbit, polyclonal Unpurified antiserum (**cat # TIE22-S**) Aff pure IgG (**cat #TIE22-A**) purified over antigen-agarose column
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)
-ve control	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Recombinant human Tie-2 protein (23-744 aa, EC domain) was expressed as His-tagged Fc protein Chimera and purified (>90%). Recombinant fusion protein (cat # TIE22-C) migrates as ~165 kDa due to glycosylation. F**or WB +ve control**, **Cat # TIE22-C**, is formulated in SDS-PAGE sample buffer (reduced). This preparation is not biologically **inactive**. It is not suitable for ELISA or other applications where native protein is required. It is supplied in 100 ul/vial. For WB, heat once and load 10 ul/lane and visualize with appropriate antibodies. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the control solution prior to heating and loading on gels. This preparation is not biologically active. It is intended for qualitative purpose and not to serve as standard of known concentration. Store frozen in suitable aliquots. Do not freeze, thaw, or heat repeatedly.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage
Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..
Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1-10 ug/ml for affinity pure using Chemiluminescence technique). Native Tie-2 is ~140 kDa. Recombinant fusion protein (cat # TIE22-C) migrates as ~165 kDa due to glycosylation.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 2-20 ug/ml in formaldehyde fixed tissue.

Specificity & Cross-reactivity

Human TIE22-P sequence is 100% conserved in rat, mice, and 94% bovine Tie-2. The peptide is specific for Tie-2 as no significant sequence homology is seem with Tie-1. Antibody crossreactivity in various species is not established. The Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at: www.4adi.com\data\abblock.html).

General References: (1) Ziegler SF (1993) Oncogene 8, 663; Vikkula M (1996) Cell 87, 1181; Sato TN (1993) PNAS 90, 12056; Dumont DJ (1993) Oncogene 8, 1293; Horita K (1992) BBRC 189, 1747; Runtig AS (1993) Growth factors 9, 99;

**This product is for in vitro research use only.*

TIE22-S-A-P-C 70322A

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