ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Anion Exchanger-1 (AE-1/Band3) Antibodies

Cat. # AE12-M Mouse Monoclonal Anti-Human Band-3/AE-1 antisera # 2, ascites **SIZE**: 100 ul

Anion exchangers (AE) are membranes proteins involved in the regulation of intracellular pH, cell volume regulation as well as in transepithelial acid/base transport. AE proteins are sodium–independent exchangers that mediates one-for-one exchange of extracellular Cl- for intracellular HCO3- ions resulting in intracellular acidification. AE proteins are encoded by a family of at least three related genes (**AE1-4**). AE proteins are exemplified by a large N-terminal cytoplasmic domain (~40-75 kDa) that provides binding sites for cytoskeleton protein, glycolytic enzymes and hemoglobin. The N-terminal cytoplasmic domains of AE2 are AE3 are more closely related than AE1. In fact, AE1 NT is 300 aa shorter than both the AE2 and AE3. The CT TM domain (~55 kDa) is highly conserved (~70% identity) among various AE, spans the lipid bilayer 12-14 times, and is able to mediate anion exchange by itself.

AE1 protein (also know as band 3) is the most abundant protein in red cell membrane. Human **AE1** gene (chromosome 17q21-qter) encodes for a protein of 911 aa (rat 927 aa; mouse 929 aa). Several isoforms of AE1 are also transcribed. AE1 is primarily expressed in red cells and kidney. AE1 is dimeric in solution. Defects in AE1/band3 gene are the cause of hereditary ovalocystosis (HO) – a disease associated with high degree of membrane rigidity.

FUNCTION: Band 3 has two functional domains: its integral domain mediates a 1:1 exchange of inorganic anions across the membrane, whereas its cytoplasmic domain provides binding sites for cytoskeletal and other proteins (By similarity).
SUBUNIT: A dimer in solution, it spans the membrane asymmetrically and appears to be tetrameric.
SUBCELLULAR LOCATION: Basolateral cell membrane; Multi-pass membrane protein. Note=In the basolateral membrane of intercalated cells of distal tubules and collecting ducts.
ALTERNATIVE PRODUCTS: 2 named isoforms [FASTA] produced by alternative splicing. Isoform 1 (erythrocytes 927 aa) and isoform 2 (kidney 848aa; missing 1-79 aa)

Source of Antigen and Antibodies

.**Antigen**	Purified human band-3/AE-1 protein was used as antigen to immunize mice. Resulting clone (IgG2a) was expanded as ascites. The epitope for this antibody is located within the 20 kDa from the N-terminus domain
Ab Host/type	Mouse, Monoclonal ascites, unpurified, (Cat # AE12-M)
2-Ab	**Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Ascites

100 ug/100ul solution lyophilized powder

Supplied in **0.05% azide**

Reconstitute powder 100 ul PBS

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 (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique). AE1 is approx. 90-100 kDa proteins. It aggregates easily forming dimers and multimers. Aggregation may be avoided if membranes are prepared in N-ethylmaleimide. It may also be proteolytically processed to smaller fragments of 60, 40, 20 kDa.

ELISA: Control AE1 protein can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested. We recommend the use of affinity purified antibody at 1-20 ug/ml in paraformaldehyde fixed sections of tissues.

Specificity & Cross-reactivity

The AE12-M monoclonal antibody does not recognize band-3 from horse, mouse, bovine, pig, g. pig, dog or mouse erythrocytes. It also does not recognize non-erythroid band-3 from human fibroblasts.

General References: 1. Kudrycki KE et al (1989) J Biol Chem. 264, 8185; Kopito RR et al (1985) Nature 316, 234; Raida M et al (1989) BBA 980, 291; Lux SE et al (1989) PNAS 86, 9089; Tanner MJ et al (1988) Biochem. J. 256, 703.

*This product is for In vitro research use only.

Related material available from ADI

Antibodies AE1-7 and CLC-K1; KCCL1-3; AQP-9 and RUT; OCT and OAT, AE-3, and NACX

Recycle your blot in Just 5-10 min. (use the same strip for various Dopamine receptors) New formulation will strip antibodies in just a few minutes at room temp. (no boiling or pungent mercaptoethanol).

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