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Antibodies to Ovarian Cancer Marker

Epididymal secretory specific Protein E4 antibodies (ESP-101AP)

Alternate Nomenclature: Epididymal secretory specific Protein E4, Protease inhibitor WAP5, HE4 or WFDC2

The whey acidic protein (WAP) domain is a conserved motif, containing eight cysteines found in a characteristic 4-disulphide core arrangement, that is present in a number of otherwise unrelated proteins. One of these proteins, which contains two WAP domains, is HE4 (also known as WFDC2), originally described as an epididymis specific protein but more recently suggested to be a putative serum tumour marker for ovarian cancer and a presumptive role in natural immunity. The HE4 protein expression is not only confined to epididymis but is expressed in a number of normal human tissues outside the reproductive system, including regions of the respiratory tract and nasopharynx and in a subset of lung tumor cell lines. HE4 gene expression was highest in normal human trachea and salivary gland, and to a lesser extent, lung, prostate, pituitary gland, thyroid, and kidney. Highest level of expression of ESPE4 was observed in adenocarcinomas of the lung, and occasional breast, transitional cell and pancreatic carcinomas (1). The WFDC2 gene undergoes extensive splicing in malignant tissues that give rise to five WAP domain containing isoforms (2).

WFDC2 is expressed in some epithelial cells of the upper airways as well as in mucous cells and ducts of submucosal glands. No staining was seen in peripheral lung. Intense staining is found in major salivary glands and in minor glands of the nose, sinuses, posterior tongue and tonsil. Studies with the related protein Secretory Leukocyte Protease Inhibitor (SLPI) show that although both proteins are expressed in similar tissues, the precise cellular localization differs. Significant increases in expression and localization of WFDC2 are seen in patients with Cystic Fibrosis (3). ESPE2 protein is a 124 amino acids (15 kDa) protein. ESPE4 protein has sequence homology to extracellular proteinase inhibitor and is localized on human chromosome 20. The ESPE4 protein is a secreted glycoprotein (Asn-44) that is over expressed in serous and endometrioid ovarian carcinomas (4). There are at least 5 named variants (HE4-V1 to HE4-V5) expressed as a result of alternate splicing and they serve as endopeptidase inhibitors.

The ESPE4-selective antibodies were generated against a peptides form near C-terminal end of the protein. FabGennix Inc. has generated epitope specific rabbit anti-ESPE4 polyclonal (epitope-specific) antibodies utilizing linear and cyclic peptide sequences. The ESPE4 antibodies are affinity purified over immobilized antigen based chromatography, and the purified immunoglobulin are stabilized in antibody stabilization buffer. Limited quantities of the antigenic blocking peptide for ESPE4 antibodies are also available (inquire for availability). *FabGennix Inc.* will also conjugate antibodies with enzymes or fluorescent probes as custom service upon request at a reasonable cost. FabGennix International Inc., has produced a number of antibodies for diagnostic use, please visit www.FabGennix.com for a complete listing.

Catalog #	Host	Description	Antigen/ control	Cross reactivity	Qty/Price
ESP-101AP	Rabbit	Affinity purified ESPE4 Antibodies	KLH conjugated peptide	R, M, H	200ul
ESP-112AP	Rabbit	Affinity purified ESPE4 antibodies (mid-region epitope)	KLH conjugated peptide	R, H, M	200ul
P-ESP	Rabbit	Antigenic blocking peptide for ESP-101AP antibody	Antigenic free peptide	R, M, H	100ul
PC-ESP	n/a	Western blot positive control for ESPE4 protein.	Protein in ready-to-use buffer	250 ug	inquire

R = rat; M = mouse; H = humans; R = rabbit * Actual volume is 103-110 µl; WB, Western Blot analyses; IMM, Immunoprecipitation; IHC, Immunohistochemistry, n.d, not determine.

Immunogen: Synthetic peptides taken from near N and C-terminal regions on the ESPE4 protein. Peptide was post-synthetically modified by attaching a linker for conjugation with KLH. The peptide was coupled to KLH using hetero-bi-functional cross linker for immunogen preparation.

Concentration: ESP-101AP = IgG concentration 0.68-0.95 mg/ml.

Applications: ELISA: Antibody dilution 1:20,000 for ELISA or DOT blot assay. W.B: Antibody dilution 1:500-750 for WB using PC-ESPE4; IMM: n.d; IHC n.d. The cross species reactivity of this antibody has not been examined in detail.

Reactivity: The antibodies ESP-101AP labels a 15 kDa protein in PC-ESPE4 samples.

Protocols: Standard protocol for various applications (Western blot; immunoprecipitation and immunohistochemistry) of this antibody can be obtained by calling Technical support line. general information on this antibody is provided with the product specification sheet, and however, FabGennix Inc. recommends investigators to optimize conditions.

Form/Storage: The antiserum is supplied in antibody stabilization buffer with preservatives. For long-term storage of antibody, store at -20°C FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi-use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µm filter after every use for long-term storage.

***Note:** Briefly centrifuge to collect liquid before opening the vial, heat the PC-ESPE4 tube in 90°C water bath for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature. Repeated freezing and thawing may result in appearance of higher molecular weight immunoreactive bands.

Note: Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripOBuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating is required.

References:

1. Galgano MT, Hampton GM, Frierson HF Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. *Mod Pathol.* 2006 Jun;19(6):847-53.
2. Bingle L, Singleton V, Bingle CD. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. *Oncogene.* 2002 Apr 18;21(17):2768-73.
3. Bingle L, Cross SS, High AS, Wallace WA, Rassl D, Yuan G, Hellstrom I, Campos MA, Bingle CD. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. *Respir Res.* 2006 Apr 6;7:61.
4. Drapkins R., Horsten v. H. H., Lin Y., et al., Human epididymis protein (HE4) is secreted glycoprotein that is over expressed by serous and endometrioid ovarian carcinomas. *Cancer Res.* 65, 2162-69, 2005.

* For users who may require large amounts of ESP-101AP and ESP-112AP, please enquire about bulk material discounts.
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

Western Blot of ESP-101 with PC-ESPE4 sample. Antibody dilution 1:500 in antibody dilution buffer (DiluOBuffer). ESPE4 approximate MW is a 15 kDa protein.

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