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## Antibodies to *Drosophila melanogaster* (Fruit Fly) TGFbeta pathway Proteins

### Anti-MEDEA protein antibodies (Cat # MEDEA-101AP)

Alternative nomenclature: human SMAD 4 like, Accession #: AAC60025 .

The fly *Drosophila melanogaster* is one of the most intensively studied organisms in biology and serves as a model system for the investigation of many developmental and cellular processes common to higher eukaryotes, including humans. Approximately 120 mb euchromatic portion of the fly genome has been sequenced using a shotgun sequencing approach. The entire fly genome encodes for approximately 13,600 genes with vast functional diversity. The dorsal ventral patterning in vertebrate embryos is regulated by members of TGF-beta family of growth and differentiation factors. The receptors for ligands in the TGF-beta superfamily are kinases but not much is known about their down stream components. In *Drosophila* the decapentaplegic protein (dpp), a homolog of vertebrate BMP2 and BMP4 is involved in dorsal ventral axial patterning. Several downstream component of dpp signaling are characterized including MAD and MEDEA that codes a unique predicted cytoplasmic with no identifiable functional domain. Phenotypic analysis of the new Medea mutations indicates that Medea, like Mad, is required for both embryonic and imaginal disc patterning. Complete elimination of maternal and zygotic Medea activity in the early embryo results in a ventralized phenotype identical to that of null dpp mutants, indicating that Medea is required for all dpp-dependent signaling in embryonic dorsal-ventral patterning (1). Two Smads have been identified in *Drosophila*. Mothers against dpp (Mad) is a pathway-specific Smad, whereas Daughters against dpp (Dad) is an inhibitory Smad genetically shown to antagonize Dpp signaling.

The protein coded by Medea has a striking sequence similarities to human SMAD4. Medea (SMAD4)AD forms heteromeric complexes with *Drosophila* SMAD4 (Medea) upon phosphorylation by thickvein (Tkv), a type 1 receptor for Dpp (2). Like dpp, Medea is essential for embryonic dorsal/ventral patterning. However, Mad is essential in the germline for oogenesis whereas Medea is dispensable (3). MEDEA is localized in the cytoplasm, is not regulated by phosphorylation, and requires physical association with MAD for nuclear translocation. The Medea protein is a 745 amino acid (MW 87-89kDa) cytosolic protein expressed during early developmental stages of fly. The mammalian counter parts of Medea is SMAD4 acting as a down-stream transcriptional activators. SMAD2 and SMAD3 are phosphorylated as a result of the canonical cascade through ligand binding and receptor kinase activation. These phosphorylated SMADs (pSMAD) associate with SMAD4, a co-SMAD, and transcriptionally activate TGFbeta-mediated genes. In *Drosophila* Medea is not phosphorylated during either development or by its physiological actions.

The Medea-selective antibodies were generated against synthetic peptides from unique regions on the C-terminal end of protein. The Mad peptide was post-synthetically modified to achieve the desired antigenicity before injecting in to rabbits to obtained antibodies. The antibodies were isolated on an immobilized antigen based affinity matrix before stabilizing them in antibody stabilization buffer. The Medea antibodies label Medea A protein at 89kDa band in PC-Medea samples. There are at least 2 more bands found in fly extracts. *FabGennix Inc.* provides Western blot positive control samples for Medea in ready-to-use buffer for SDS-PAGE and western blotting applications. Medea positive control appears as a diffuse band at 89kDa. *FabGennix International Inc.*, has also produced antibodies to fruit fly TGFbeta pathway targets, for a complete listing please visit [www.FabGennix.com](http://www.FabGennix.com). Limited quantities of antigenic blocking peptide for Medea antibody is also available (Inquire before placing orders). For a complete listing of all *Drosophila* related antibodies, visit our website at [www.FabGennix.com](http://www.FabGennix.com). *FabGennix International Inc.*, will conjugate and couple these antibodies to fluorescent probes and secondary enzymes at a nominal price.

Catalog #	Description	Host	Cross reactivity	Qty
Medea-101AP	Medea affinity purified antibodies	Rabbit	Fruit Fly	100 ug
P-Medea	Antigenic blocking peptide for Medea antibody	Synthetic peptide	n/a	250ug
PC-Medea	Western blot positive control for Medea	Partially purified protein	Fruit Fly	For 5 appl

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

**Immunogen:** Medea antibodies are developed against conserved antigenic peptide sequences corresponding to amino acid 194-209, the peptide (ygp pgg pse yvg dan p) was covalently modified to achieve desired antigenicity before used as antigen.

**Concentration:** Antibodies Mad-101AP has immobilized antigen based affinity chromatography purified immunoglobulin concentration of 0.5-0.73mg/ml in antibody stabilization buffer.

**Applications:** ELISA/dot blot: Antibody dilution 1:20,000-1:50,000. Western blot: Antibody dilution 1:500-750 in DiluOBuffer. IMM: n.d; IHC n.d

**Reactivity:** The antibodies Medea (Medea-101AP) labels 89kDa Medea protein in PC-Medea samples.

**Protocols:** Description and use of this antibody in various applications is provided with the product. Standard protocol for various applications (Western blot; immunoprecipitation and immunohistochemistry) of this antibody can be requested by calling our Technical support line. The recommended dilutions are for reference only and *FabGennix Inc.* strongly recommends investigators to optimize conditions for use of this product in their laboratories.

**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 0.45um filter after every use for long-term storage.

#### References:

- Hudson JB, Podos SD, Keith K, Simpson SL, Ferguson EL, Thomsen GH. The *Drosophila* Medea gene is required downstream of dpp and encodes a functional homolog of human Smad4. *Xenopus* mothers against decapentaplegic is an embryonic ventralizing agent that acts downstream of the BMP-2/4 receptor. *Development*. 1998 Apr;125(8):1407-20.
- Inoue H, Imamura T, Ishidou Y, Takase M, Udagawa Y, Oka Y, Tsuneizumi K, Tabata T, Miyazono K, Kawabata M. Interplay of signal mediators of decapentaplegic (Dpp): molecular characterization of mothers against dpp, Medea, and daughters against dpp. *Mol Biol Cell*. 1998 Aug;9(8):2145-56.

\*Note: Briefly centrifuge to collect liquid, heat or boil PC-Mad tube 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.

Now Western blots can be stripped and recycle using our specially formulated StripOBuffer (Cat # FGI-1989) up to 8 times with out any distortion and significant loss in signal to noise ratios. This stripping buffer does not require heating or have any pungent smell.

\* For users who may require large amounts of Mad-101AP, 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 PC-Medea with Medea-101AP. Antibody dilution was 1:500 in diluOBuffer.

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