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### Prostaglandin E2 Receptor (EP3) selective antibodies

#### Variant-selective anti-Prostaglandin E2 Receptor (EP3) type 2 (EP3-II) Antibodies (EP3-320P and EP3-321AP)

Eicosanoids exert their cellular action through specific G-protein coupled receptors. Prostanoids are the products of cyclooxygenases action on C-20 unsaturated fatty acids that are released by action of phospholipase A2 from cell membranes in response to various stimuli. Prostaglandin E2 is the major prostanoid produced as a consequence of inflammation. Various PGE2 mediate defects on smooth muscle, vascular smooth muscle, muscle tone, renal salt and water re-absorption and rennin release has been described. Rapid progress has been made in cloning of members of the family of eicosanoid receptors. Kidney is the major site of arachidonic acid release and its subsequent enzymatic conversion to bioactive prostanoids via cyclo-oxygenase metabolic pathway. PGE2 is the only prostanoid described for which there are four E-Prostanoid receptors (designated EP1, EP2, EP3 and EP4) which have been shown to mediate a variety of effects of prostaglandin E2 (PGE2) on kidney glomerular hemodynamics, tubular salt and water reabsorption and on renal vasculature (1). EP3 receptors cause smooth muscle contraction in chick ileum and inhibit acid secretion in rat gastric mucosa, are activated by both sulprostone and AY 23636, but are not antagonized by SC19220 (2). EP3 receptors are negatively coupled to adenylate cyclase and upon activation decrease the cellular levels of cAMP (3). The EP3 have multiple variants that are expressed in glomeruli, Tamm-Horsfall negative late distal convoluted tubules, connecting segments, cortical and medullary collecting ducts as well as in the media and endothelial cells of arteries and arterioles (2).

The EP3 receptor protein is approx. 55 kDa (405 amino acids) protein which has 7 transmembrane domains, characteristic of G-protein coupled receptors. The EP3 sequence analyses suggest the presence of 7 transmembrane domains, a characteristic feature of GPCR. The protein has putative N-glycosylation sites near the extra cellular N-terminal end of the proteins. The protein has a large 3<sup>rd</sup> intra cellular loop which might be the site for the interaction of G-proteins. The short carboxy terminal is intracellular and has putative post-translational sites.

The Anti-EP3II-selective antibody was generated against a conserved sequence near the C-terminal that is unique to EP3II protein only. The polyclonal antibody strongly labels a 55-60 kDa protein in PC-EP3II samples. Anti-EP3II-selective antibody is also available in affinity-purified form for Immunoprecipitation, immunohistochemistry and Western blotting analyses. *FabGennix Inc.* will also conjugate antibodies with fluorescent probes upon request at extra charge. *FabGennix Inc.* also provides antibodies against several other GPCRs including Dopamine D1 and D2 receptors, chemokine receptors, and orphan receptors. *FabGennix Inc.*, employs cyclic peptide methodology for generating antibodies, which results in higher titer and specificity (2). *FabGennix, Inc.*, will also provide Western blot positive controls for most of these antibodies in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigenic peptides are also available. Please enquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	price
EP3-320P	Rabbit	Polyclonal antisera	R, M, H	100 µl	205
EP3-321AP	Rabbit	Affinity purified IgG	R, M, H	100ug	225
P-EP3II	N/A	Antigenic peptide	human, rat seq	500ug	95
PC-EP3II	n/a	Western blot positive control	R, M	5 appl	185

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; \* For 5 applications

**Immunogen:** Synthetic peptide (selective for EP3II receptor +C-terminal region) →

**Concentration:** EP3-320P serum (100 ul); EP3-321AP, IgG concentration 0.5-0.6 mg/ml in antibody stabilization buffer.

**Applications:** Antibody EP3-300; EP3-321AP antibodies are ideal for WB. IMM, IHC assays have not been standardized. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay. WB: > 1:500; IMM & i.p pull-down assays: n.d  
55 kDa EP3 Receptor

**Reactivity:** This antibody detects a single 55kDa EP3 protein in our western blot positive control samples (Catalog # PC-EP3II). The antibody does not cross reacts with any other EP receptors.

**Protocols:** Standard protocol for various applications (WB, IMM, IHC) of this antibody is provided with the product specification sheet, however, *FabGennix Inc.* strongly recommends investigators to optimize conditions for its use.

**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide as preservative. The affinity-purified antibodies are isolated on immobilized antigen-affinity based chromatography. For long-term storage of antibodies, store at -20oC. *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 0.45u filter after every use for long-term storage.

**References:**

1. Morath. R., Klein T., Seyberth H., Nusing. R. M. J. Am. Soc. Nephrology 10, 1851-1866, 1999.
2. Kotani M., Tanaka I., Usai T et. al., Mol. Pharmacol. 48, 869-879, 1995.
3. Farooqui, S. M., Brock. W. J., A. Hamdi., Prasad. C. (1991) J. Neurochem. 57, 1363-1369.

\* For users who may require large amounts of EP3-320P or EP3-321AP, please enquire about bulk material discounts.

This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

011502-0020SF1007Z-rev10.1

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