3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Monoclonal Anti-Activin A β_A subunit
Clone 69403.11
produced in mouse, purified immunoglobulin

Catalog Number **A1719**

Product Description

Monoclonal Anti-Activin A β_A subunit (mouse IgG1 isotype) is derived from a murine hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with purified mature recombinant human activin A, expressed in CHO cells. The antibody is purified from ascites fluid using protein G.

Monoclonal Anti-Activin A may be used to neutralize the bioactivity of recombinant human activin A. It also recognizes activin A precursors. The antibody recognizes activin A β_A subunit and inhibin A β_A subunit by capture ELISA.

Activin-A β_A subunit is a disulfide-linked dimeric protein secreted by Sertoli cells in the testis and grandulosa cell in the ovary. In the early studies, this peptide was thought to be an inhibin and not recognized as a unique compound[4,5]. Activins and inhibins have been furthur characterized and include 3 separate peptides exhibiting a combination of +α, β_A, and β_B subunits. Recently the C, D, and E-β subunits have also been cloned[4]. Activins are homodimers or heterodimers made up of the β subunit isoforms. Mammalian activin-A is identified as the $\beta_A\beta_A$ form. Bovine, porcine, human, and murine activin-A demonstrate 98% homology. These compounds are classified as members of the TGF-β super family due to amino acid homology with respect to the conservation of 7 of the 9 cysteine residues common to all TGF-β forms[4].

Activin-A has been recognized for its range of activities involving growth and differentiation of several tissues from different species [1,2]. It plays a key role in production and regulation of hormones such as FSH, LH, GnRH, and ACTH. Activin also influences erythropoiesis and the potentiation of erythroid colony formation, oxytocin secretion, paracrine, and autocrine regulation[4].

Reagent

Supplied lyophilized from a 0.2 μm filtered solution of phosphate buffered saline with 5% trehalose.

Endotoxin level is 0.1 EU per μg antibody as determined by the LAL method.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 μm-filtered PBS to produce a 0.5 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing

Procedure

Neutralization of Bioactivity

Human Activin A induces hemoglobin expression in K562 cells in a dose dependent manner. To measure the ability of the antibody to neutralize the bioactivity of human activin A, recombinant human activin A was incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this preincubation period, K562 cells were added. The assay mixture in a total volume of 200 μL per well, containing antibody at the concentrations indicated (0.01 μg/ml-100 μg/ml), recombinant human activin A at 7.5 ng/ml, and cells at 2.5×10^4 cells/ml were incubated at 37 °C for 4days in a humidified CO_2 incubator. At the end of the incubation, the hemoglobin level in cell lysate was measured for its pseudoperoxidase activity.

The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

Product Profile

Neutralization: a working antibody concentration of 0.02-0.06 μg/mL will neutralize 50% of the bioactivity due to 7.5 ng/ml recombinant human activin A using K562 cells.

Capture ELISA: the antibody can be used as the capture antibody in a human Activin A ELISA in combination with biotinylated, Actvin A monoclonal detection antibody. Using plates coated with 100 μL/well of the capture antibody at 0.5 μg/mL, in combination with 100 μL/well of the detection antibody, an ELISA for sample volumes of 100 μL can be obtained. Titrate each preparation of the recombinant protein for standard preparation to arrive at the most suitable dose range. For this ELISA, a two-fold dilution series of the protein standard starting at 8 ng/mL is suggested.

Note: In order to the obtain best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Smith, J., et al., Expression of a Xenopus homolog of Brachyury (T) is an immediate-early response to mesoderm induction. *Cell*. **67**, 79 (1991).
2. de Winter, J., et al., Activin is produced by rat Sertoli cells in vitro and can act as an autocrine regulator of Sertoli cell function. *Endocrinology*, **132**, 975 (1993).
3. Sporn, M.B., and Roberts, A.B., eds., Peptide Growth Factors and Their Receptors, Springer-Verlag Heidelberg, Vol I, p429 (1991).
4. Sporn, M.B., and Roberts, A.B., eds. Peptide Growth factors and Their Receptors, Springer-Verlag Heidelberg, Vol II, pp 217-235 (1991).
5. De Jong, F., et al., Effects of factors from ovarian follicular fluid and Sertoli cell culture medium on in-vivo and in-vitro release of pituitary gonadotrophins in the rat: an evaluation of systems for the assay of inhibin. *J. Reprod. Fertil*., **26**, 47 (1979).
6. Woodruff, T.K., Regulation of cellular and system function by activin. *Biochem. Pharmacol*., **55**, 953-963 (1998).
7. Ying, S.Y., et al., Activins and activin receptors in cell growth. *Proc. Soc. Exp. Biol. Med*., **214**,114-22 (1997).
8. Vale, W. et al., The Inhibin/Activin Family of Hormones and Growth Factors." In: Peptide Growth factors and their Receptors. Sporn, M., Roberts, A., eds., Berlin, Springer-Verlang. pp. 211-248 (1990).
9. Kingsley, D.M., The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes Dev*., **8**, 133 (1994).
10. Halvorson, L.M. and DeCherney, A.H., Inhibin, activin, and follistatin in reproductive medicine. *Fertil. Steril*. **65**, 459 (1996).

KEK,PHC 01/10-1