

## Product Information

### **MONOCLONAL ANTI-ACTIVIN A ( $\beta_A$ SUBUNIT), HUMAN, CLONE 69403.11**

Purified Mouse Immunoglobulin

Product Number **A1719**

#### **Product Description**

Monoclonal Anti-Activin A ( $\beta_A$  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 ( $\beta_A$  subunit) and inhibin A ( $\beta_A$  subunit) by immunoblotting and ELISA.

Monoclonal Anti-Activin A may be used for neutralization, ELISA and immunoblotting.

Activin-A ( $\beta_A$  subunit) is a disulfide-linked dimeric protein secreted by Sertoli cells in the testis and granulosa cell in the ovary. In the early studies, this peptide was thought to be an inhibin and not recognized as a unique compound<sup>4,5</sup>. Activins and inhibins have been further characterized and include 3 separate peptides exhibiting a combination of  $+\alpha$ ,  $\beta_A$ , and  $\beta_B$  subunits. Recently the C, D, and E- $\beta$  subunits have also been cloned<sup>4</sup>. Activins are homodimers or heterodimers made up of the  $\beta$  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- $\beta$  super family due to amino acid homology with respect to the conservation of 7 of the 9 Cysteine residues common to all TGF- $\beta$  forms<sup>4</sup>.

Activin-A has been recognized for its range of activities involving growth and differentiation of several tissues from different species<sup>1,2</sup>. 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<sup>4</sup>.

#### **Reagents**

Monoclonal Anti-Activin A is supplied lyophilized from a 0.2  $\mu$ m filtered solution of phosphate buffered saline. Endotoxin level is < 10 ng per mg antibody as determined by the LAL method.

#### **Preparation Instructions**

To one vial of lyophilized powder, add 1 ml of 0.2  $\mu$ m-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  $-20^\circ\text{C}$ . Reconstituted product may be stored at  $2-8^\circ\text{C}$  for at least one month. For prolonged storage, freeze in working aliquots at  $-20^\circ\text{C}$ . 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  $37^\circ\text{C}$  in a 96 well plate. Following this preincubation period, K562 cells were added. The assay mixture in a total volume of 200  $\mu$ l per well, containing antibody at the concentrations indicated (0.01  $\mu$ g/ml-100  $\mu$ g/ml), recombinant human activin A at 7.5 ng/ml, and cells at  $2.5 \times 10^4$  cells/ml were incubated at  $37^\circ\text{C}$  for 4 days in a humidified  $\text{CO}_2$  incubator. At the end of the incubation, the hemoglobin level in cell lysate was measured for its pseudoperoxidase activity. The  $\text{ND}_{50}$  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**

For neutralization, a working concentration of 0.02-0.06  $\mu$ g/ml of Monoclonal Anti-Activin A will neutralize

50% of the bioactivity due to 7.5 ng/ml recombinant human activin A using K562 cells.

For indirect ELISA, a working concentration of 0.5-1.0 µg/ml is determined to detect a limit of ~1.6 ng/well of recombinant human activin A ( $\beta_A$  subunit) and ~3.1 ng/ml of recombinant human inhibin A ( $\beta_A$  subunit).

For indirect immunoblotting, a working concentration of 1-2 µg/ml is determined using human activin A ( $\beta_A$  subunit) at 5 ng/lane under non-reducing conditions, and using human inhibin A ( $\beta_A$  subunit) at 50 ng/lane under non-reducing conditions.

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

#### References

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