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ProductInformation

ANTI-6CKINE, HUMAN
Developed in Goat, Affinity Isolated Antibody

Product Number **C 6104**

Product Description

Anti-6Ckine is developed in goat using purified recombinant human 6Ckine expressed in *E. coli* as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-6Ckine antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide. .

Anti-6Ckine recognizes recombinant human 6Ckine by various immunochemical techniques including immunoblotting, neutralization, and ELISA. The antibody exhibits less than 15 % cross-reactivity with recombinant mouse 6Ckine and less than 5 % cross-reactivity with recombinant human fractalkine, Teck, MCP-3, mouse CRG-2, human VIC, and mouse VIC in ELISA assays.

Human 6Ckine is a β- or C-C chemokine identified in the Expressed Sequence Tag (EST) database by three independent groups.[1,2,3] Known also as Exodus-2 and secondary lymphoid-tissue chemokine (SLC), 6Ckine contains four conserved cysteine (C) residues which are characteristic of β-chemokines.[1,2,3] Two additional conserved cysteine residues have been found in its unusually long carboxy-terminal domain and consequently the name 6Ckine.[1,2,3]

Human and mouse 6Ckine are highly conserved and show 86% amino acid homology.[1] Human 6Ckine cDNA encodes a 134 amino acid precursor protein, a 23 amino acid signal peptide and a 111 amino acid mature protein.[1,3] This protein shares 21 % to 33 % homology with other human C-C chemokines.[1] Comparatively, mouse 6Ckine cDNA encodes a 133 amino acid precursor protein, a 23 amino acid signal peptide and a 110 amino acid mature protein.[1] Recombinant human 6Ckine has a predicted molecular mass of approximately 12 kDa (mature protein). In SDS-PAGE under reducing and non-reducing conditions, the recombinant protein migrates with an apparent molecular mass of 16 kDa to 17 kDa.

The human 6Ckine gene has been mapped to chromosome 9p13.[3,5] The expression of human 6Ckine has been detected primarily in lymphoid tissues but also in the gastrointestinal tract.[2,3,4,5,6] Recombinant human 6Ckine is chemotactic for some human T-cell lines, resting peripheral blood lymphocytes, and normal cultured T-cells treated with PHA and IL-2 [3,4]. Unlike other C-C chemokines, 6Ckine is not chemotactic for monocytes and neutrophils.[1,3] A growing body of work suggests that 6Ckine influences lymphocyte homing to secondary lymphoid organs [6], integrin-mediated lymphocyte adhesion [7], and may act via the EBI1 ligand chemokine (ELC) receptor, CCR7 [4,5].

Reagent

Anti-6Ckine supplied as approximately 100 µg of antiserum lyophilized from a 0.2 µm filtered solution in phosphate buffered saline (PBS).

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate-buffered saline (PBS) to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability

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

Procedure

Anti-6Ckine neutralizes the bioactivity of recombinant human 6Ckine. To measure this biological activity, recombinant human 6Ckine is incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation period, 35 µl of the cytokine-antibody solution (containing recombinant human 6Ckine at a final concentration of 0.5 µg/ml and antibody at concentrations from 0.01 to 10 µg/ml) is transferred to

the lower compartment of a 96 well chemotaxis chamber. The chemotaxis chamber is then assembled using a PVP-free polycarbonate filter (5 micron pore size) and 2 x 10^6 cells/ well (cultured human lymphocytes) are added to the top chamber. After incubation for 3 hours at 37 °C in a 5 % CO_2 humidified incubator, the chamber is disassembled and the cells that have migrated through to the lower chamber are transferred to a working plate and stained using MTT. Absorbance at 540 nm is read on a microplate reader.

The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is defined as that concentration required to yield one-half maximal inhibition of the 6Ckine activity on a responsive cell line, when 6Ckine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize recombinant human 6Ckine activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity.

Product Profile
The Neutralization $Dose_{50}$ (ND_{50}) for this antibody is approximately 4 to 8 µg/ml in the presence of 0.5 µg/ml of recombinant human 6Ckine, using cultured lymphocytes in a chemotaxis assay.

For immunoblotting, a working concentration of 0.1 to 0.2 µg/ml detects human 6Ckine at approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working concentration of 0.5 to 1.0 µg/ml detects recombinant human 6Ckine.

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

Endotoxin: <10 ng/mg antibody determined by the LAL method.

References

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