ProductInformation

ANTI-SDF-1β/PBSF, HUMAN
Developed in Goat, Affinity Isolated Antibody

Product Number **S 2687**

Product Description

Anti-Stromal Cell-Derived Factor 1β (SDF-1β)/Pre-B Cell Growth Stimulating Factor (PBSF) is developed in goat using purified recombinant human SDF-1β/PBSF expressed in *E. coli* as immunogen. Recombinant Human Stromal Cell-Derived Factor 1β (SDF-1β)/Pre-B Cell Growth Stimulating Factor (PBSF) is a 72 amino acid (~8 kDa) polypeptide expressed in *Escherichia coli*.[1, 2] Affinity isolated antigen specific antibody is obtained from goat anti- SDF-1β/PBSF antiserum by immuno-specific purification. SDF-1β specific IgG is first purified by passing the goat sera over SDF-1α affinity column. The unbound fraction from the SDF-1α affinity column is then purified using SDF-1β affinity column.

Anti- SDF-1β/PBSF recognizes recombinant human SDF-1β/PBSF by various immunochemical techniques including immunoblotting, neutralization, and ELISA. By immunoblotting, this antibody exhibits less than 5 % cross-reactivity with recombinant human SDF-1α. This antibody neutralizes 60 % to 80 % of the bioactivity of SDF-1β and does not neutralize the biological activity of SDF-1α.

SDF-1β and SDF-1α were initially identified using signal sequence trap cloning.[1, 2, 3] With this method, cDNAs have been cloned using mouse bone marrow stromal cell lines and human stromal cell lines as cytokines that support the proliferation of a stromal cell-dependent pre-B-cell line.[1, 2, 3] SDF-1 is highly conserved among species with only one amino acid substitution between the human and mouse proteins and 92% homology.[1] SDF-1β and SDF-1α (together known as SDF-1/PBSF) are encoded by a single gene and arise from alternative splicing. The two proteins are identical except for the four amino acid residues that are present in the carboxy-terminus of SDF-1β and absent from SDF-1α. The amino acid sequence of SDF-1 identifies the protein as a member of the CXC family of chemokines (α subfamily) that lacks the ELR domain. Unlike other chemokine α and β subfamily members that cluster on chromosomes 4 and 17, respectively, the gene for SDF-1 has been mapped to chromosome 10q11.1.

SDF-1 functions as a pre-B cell growth-stimulating factor in the presence of IL-7 and a potent chemo-attractant for T-lymphocytes and monocytes, but not neutrophils.[2, 4] It is also a ligand for CXCR4 (fusin/LESTR) receptor that functions as a co-receptor for lymphocyte-tropic HIV-1 strains.[4, 5] By signaling through the receptor, SDF-1 may serve as an inhibitor of HIV-1 which utilizes the LESTR/fusin receptor as a point of entry.[4] SDF-1, unlike other chemokine family members, has been found to be constitutively expressed in a wide variety of tissues including pancreas, spleen, ovary, and small intestine.[1, 2, 3]

Reagent

Anti- SDF-1β/PBSF is supplied as 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.

Product Profile

Anti-SDF-1β/PBSF neutralizes the chemoattractant activity of recombinant human SDF-1β/PBSF. To measure biological activity, recombinant human SDF-1β/PBSF is incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation, 35 µl of the cytokine-antibody solution (containing recombinant human SDF-1β/PBSF at a final concentration of 0.06 µg/ml and antibody at concentrations from 0.01 to 100 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 anti-SDF-1β is approximately 5 to 15 μg/ml in the presence of 0.06 μg/ml of recombinant human SDF-1β, using cultured human lymphocytes in a chemotaxis assay.

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

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

For immunoblotting, the recommended working concentration of the antibody is 0.1 to 0.2 μg/ml. The detection limit for human SDF-1β is approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, the recommended working concentration of the antibody is 0.5 to 1.0 μg/ml. The detection limit for human SDF-1β is approximately 0.6 ng/ml.

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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2. Nagasawa, T. et al., Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc. Natl. Acad. Sci. USA, **91,** 2305-2309 (1994).
3. Tashiro, K. et al., Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. Science, **261,** 600-603 (1993).
4. Bleul, C., et al., The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature, **382,** 829-833 (1996).
5. Oberlin, E. et al., The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature, **382,** 833-835 (1996).

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