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ProductInformation

DAN/Fc Chimera

Human, Recombinant Expressed in NSO cells

Product Number D 2316

Product Description

Recombinant Human DAN/Fc Chimera is produced from a DNA sequence encoding human DAN¹ (Met 1 - Asp 180) fused to the Fc region of human IgG1 via a polypeptide linker. The chimeric protein is expressed in mouse myeloma NSO cells. Recombinant mature human DAN/Fc is a disulfide-linked omodimeric protein. Based on N-terminal sequencing, the recombinant human DAN/Fc protein has ALA 17 at the N-terminus. The reduced monomer has a calculated molecular mass of 44.3 kDa. As a result of glycosylation, the recombinant protein migrates as a 55-65 kDa protein in SDS-PAGE under reducing conditions.

DAN (differential screening-selected gene aberrative in neuroblastoma) was originally identified as a gene whose expression is down-regulated in *src*-transformed rat fibroblasts.² Human DAN was isolated from a normal lung cDNA library using mouse DAN as a probe. DAN has now been shown to be a prototypical member of the DAN family of secreted glycoproteins that are putative antagonists for the TGF-β superfamily.

Members of the DAN family share a cysteine-rich domain that is structurally related to the cysteine-knot motif found in the TGF-β superfamily ligands.³ There are at least five mammalian DAN family members including DAN, Gremin/DRM, Cer1 (Cerberus-related), Dante, and PRDC (protein related to DAN and cereberus). Additional DAN family members include *Xenopus* Cerberus, chick Caronte, and *C. elegans* CeCan 1.

The DAN family of proteins are thought to act as antagonists by binding TGF- β family ligands and preventing their interactions with signaling receptor complexes. It is likely the various DAN family members and other TGF- β /BMP antagonists (Noggin, Chordin, Follistatin, and TSG) can selectively antagonize the activities of different subsets of TGF- β family ligands. These antagonists represent one of the many regulatory mechanisms that have evolved to control the bioactivities of the TGF- β superfamily ligands.

Reagent

Recombinant Human DAN/Fc Chimera is supplied as approximately 50 μg of protein lyophilized from a 0.2 μm filtered solution in phosphate buffered saline.

Preparation Instructions

Reconstitute the contents of the vial using 0.2 μ m filtered phosphate buffered saline containing at least 0.1% human serum albumin or bovine serum albumin. Prepare a stock solution of no less than 10 μ g/ml.

Storage/Stability

Store at –20 °C. Upon reconstitution, the product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Do not store in a "frost-free" freezer.

Product Profile

Recombinant Human DAN/Fc Chimera is measured by its ability to inhibit BMP-4 activity in ATDC-5 chrondrogenic cells.

The ED $_{50}$ for this effect is typically 1.5-6 μ g/ml in the presence of 30 ng/ml of recombinant human BMP-4 (Prod. No. B 2680).

Purity: > 95% as determined by SDS-PAGE, visualized by silver stain.

Endotoxin level is < 1.0 EU (endotoxin units) per 1 μ g of cytokine as determined by the LAL (*Limulus* amebocyte lysate) method.

References

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- Ozaki, T., and Sakiyama, S., Molecular cloning and characterization of a cDNA showing negative regulation in *v-src*-transformed 3Y1 rat fibroblasts. Proc. Natl. Acad. Sci. USA, 90, 2593-2597 (1993).
- 3. Stanley, E., et al., DAN is a secreted glycoprotein related to *Xenopus* cerberus. Mech. Dev., **77**, 173-184 (1998).
- 4. Massague, J., and Chen, Y-G., Controlling TGF- β signaling. Genes & Dev., **14**, 627-644 (2000). kaa 02/03