C-X-C - Chemokine Receptor Type 4 # PL016

Product specification

Acronym: CXCR4
Origin species : Human
Protein reference : P61073 (UniProtKB)
L01639.1 (GenBank)
Family: GPCR

Expression system: E.coli based CFPS
Format: Proteoliposomes
Protein sequence: Met1 – Ser352
Tag : 6xHis tag (N-terminal)
Cleavage site: Factor Xa
Product MW: 43 kDa
Application: Drug screening & discovery, antibody development, structural biology

Product description

Human CXCR4 (C-X-C Chemokine Receptor type 4) is an α-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12), a molecule endowed with potent chemotactic activity for lymphocytes. CXCR4 is essential for proper fetal development and is the major co-receptor for T-tropic strains of human immunodeficiency virus 1 (HIV-1). Additionally, SDF-1 and CXCR4 mediate cancer cell migration and metastasis. The N-terminal domain of CXCR4 is the binding site for SDF-1.

Recombinant protein sequence

His tag – factor X cleavage site -
MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFV
ITLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWIPALLLTIPDFIFAN
VSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSCYCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILL
EIIKQGCEFENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKGKRGGHSSVSTESESSSFHSS

Quality analysis

Purity:
Liposomes are directly incorporated into the Cell-Free reaction, thus, some impurities from the *E.coli* lysate might be present in the proteoliposomes.
A negative control (proteoliposomes without the protein of interest) can be provided (useful for screening, immunization…).
The purity can be improved by protein expression in detergent and relipidation after purification step(s).

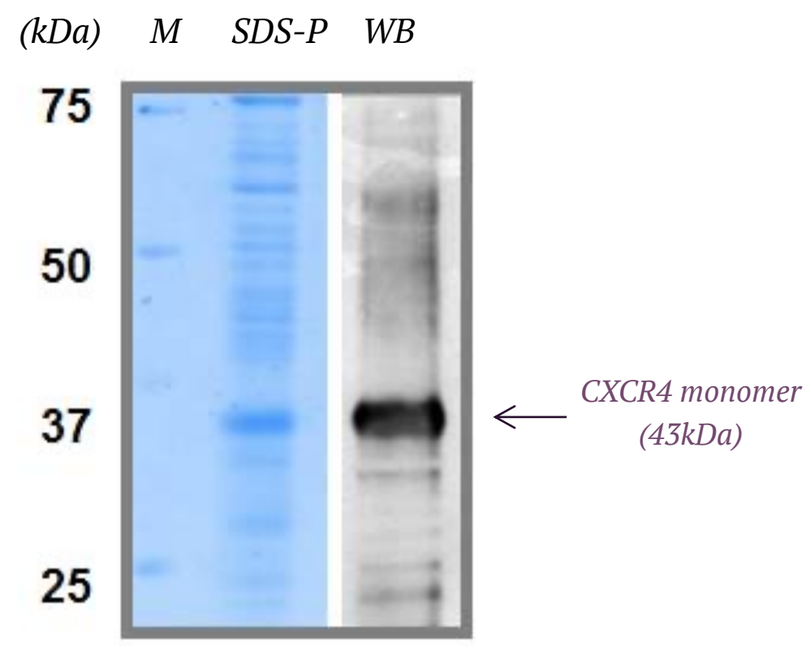


Fig.1: *Identification of CXCR4 in proteoliposomes by SDS-PAGE and Coomassie staining (SDS-P) and Western Blot (WB) using an anti-6xHis antibody.*

Purification procedure: CXCR4 proteoliposomes are purified on a sucrose gradient.

NB : Migration of membrane proteins on SDS-PAGE can results in « gel shifting » due to the presence of hairpins (helix-loop-helix)[1-3].

References :
1 – Rath A., et al., Detergent binding explains anomalous SD-PAGE migration of membrane proteins PNAS, 2009 Feb 10, vol. 106
2 – Rath A., et al., Acrylamide concentration determines the direction and magnitude of helical membrane protein gel shifts, PNAS, 2013 Sep 24, 110(39)
3 – Rath A., et al., Correction factors for membrane protein molecular weight readouts on sodium dodecyl sulfate-polyacrilamide gel electrophoresis, Anal. Biochem., 2013 Mar 1, 434(1)

Quality controls

Methods: SPRi and Cisbio Bioassays Tag-lite® technology.

The binding properties of CXCR4 proteoliposomes with SDF1α natural ligand have been validated using Horiba Scientific SPRi platform and Cisbio Bioassays Tag-lite® technology.

Results:

SPRi technology

The binding properties of CXCR4 proteoliposomes have been validated using a SPRi-Plex instrument (Horiba Scientific). The ligands were injected on a biochip grafted with CXCR4 biotinylated proteoliposomes. Specific interactions between CXCR4 proteoliposomes and its ligand SDF1-α were detected (see related Application Note). The signal was dose dependent. No signal was observed on the negative control spots (non-relevant proteoliposomes).

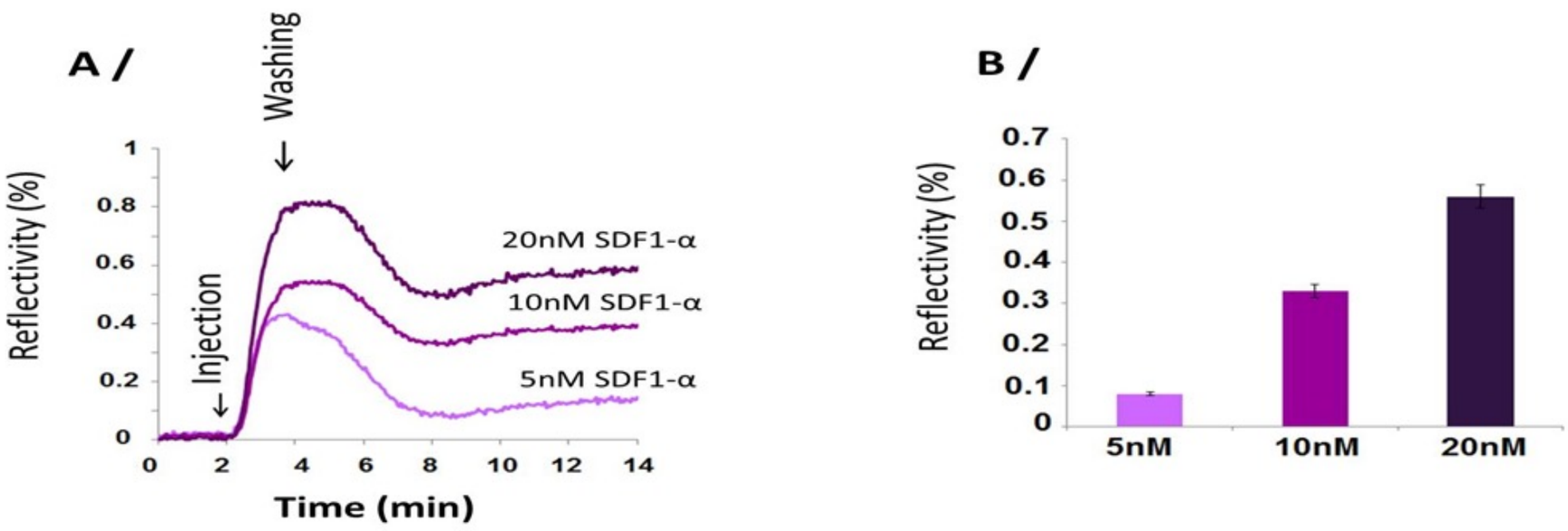


Fig.2: SPRi analysis of the CXCR4-SDF1a interaction*; A /Sensorgrams obtained after SDF1a injections; B/ Variations of reflectivity obtained at steady state. The SDF1a peptide was injected successively at different concentrations: 5nM, 10nM then 20nM*

Cisbio Bioassays Tag-lite® technology

CXCR4 binding properties were validated by Cisbio Bioassays Tag-lite technology. HEK293 Tag-Lite cell line overexpressing CXCR4 was used in a competitive binding assays with a constant SDF1α concentration against increasing concentrations of proteoliposomes. CXCR4 proteoliposomes are able to compete against the Tag-lite cell line for SDF1α ligand.

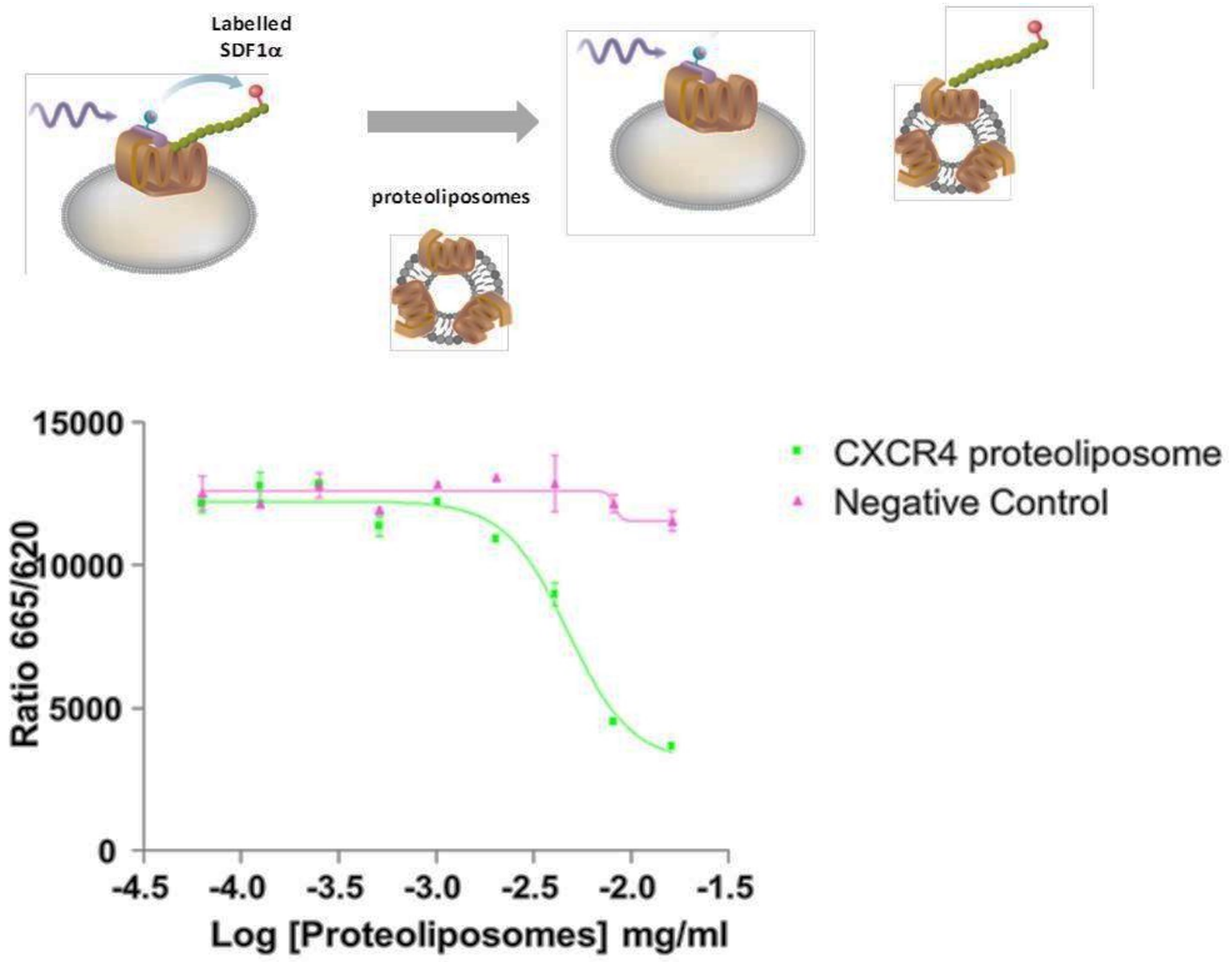


Fig.3: Competition assay between HEK293 Tag-Lite cells overexpressing CXCR4 and CXCR4 proteoliposomes

Formulation

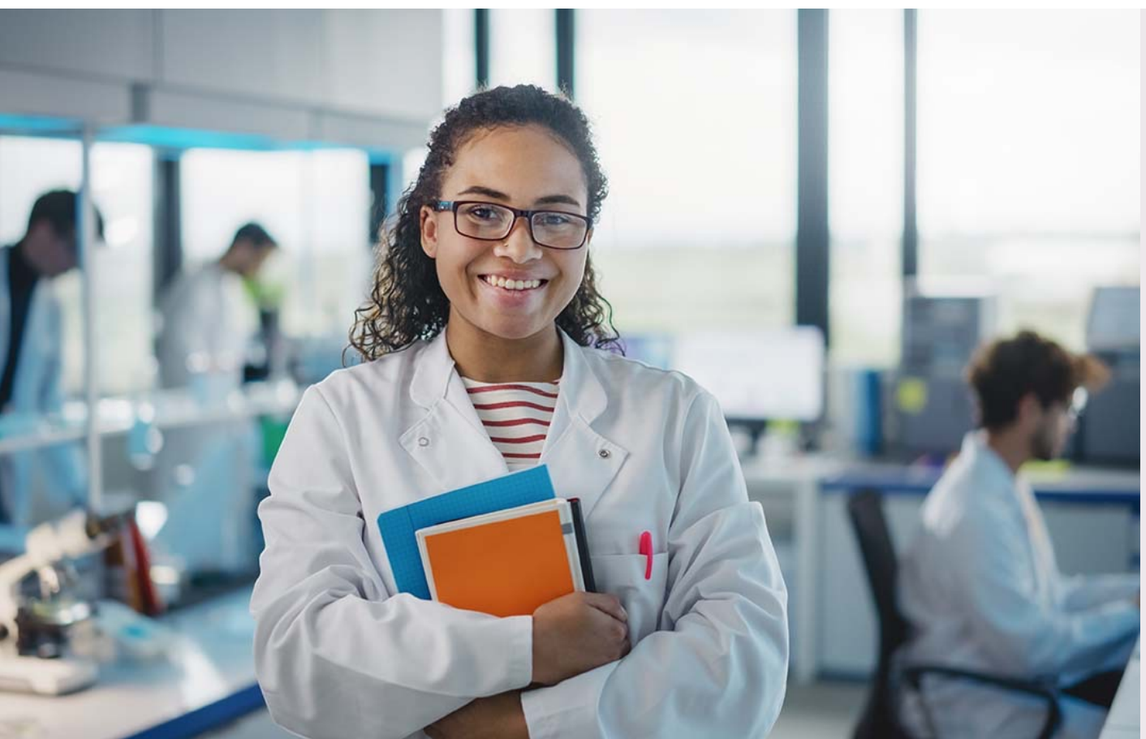
Buffer: Available in Hepes 50mM, pH 7.5 with cryoprotectants. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage.
Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 10 µg, 50 µg, 100 µg, customized quantity on request.



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