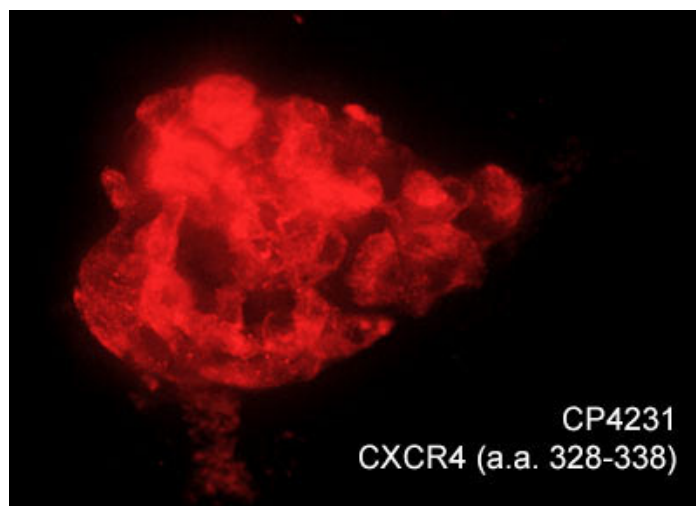


## Background

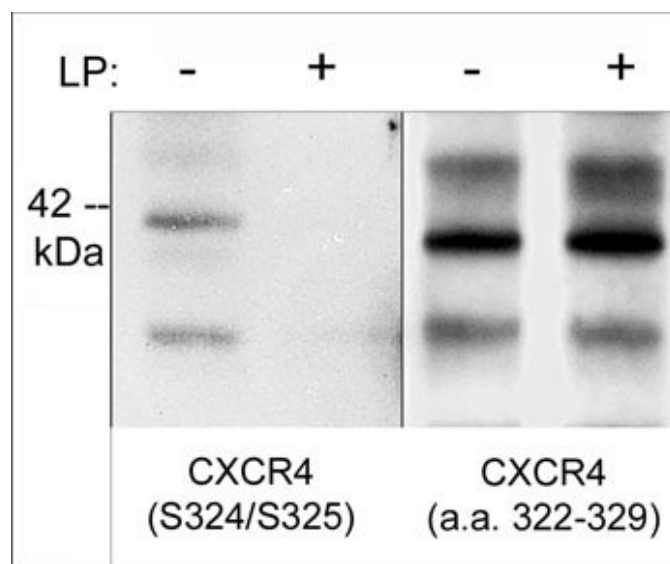
The chemokine receptor CXCR4 is a widely expressed G protein-coupled receptor required for development, hematopoiesis, organogenesis, and vascularization. In disease, CXCR4 has been implicated in WHIM syndrome, HIV, and cancers. Regulation of CXCR4 function occurs through phosphorylation at multiple sites in the C-terminal region. These sites have been shown to be phosphorylated after CXCL12 stimulation, and involve several kinases, such as PKC and GRK kinases. After CXCL12 stimulation of HEK293 cells, Ser-324 and Ser-325 become phosphorylated by PKC and GRK6, while Ser-330 and Ser-339 are phosphorylated by only GRK6. In human astroglia cells, Ser-324 and Ser-325 are rapidly phosphorylated in endogenous CXCR4, while Ser-330 was phosphorylated with slower kinetics. In addition, arrestin binding to CXCR4 is driven by this phosphorylation of far C-terminal residues. Thus, site-specific phosphorylation of CXCR4 may be regulated by multiple kinases lead to complex regulation of CXCR4 signaling.

## Background References

- Orsini, M.J. et al. (1999) *J Biol Chem.* 274(43):31076.  
 Orsini, M.J. et al. (2000) *J Biol Chem.* 275(33):25876.  
 Bhandari, D. et al. (2009) *Mol Biol Cell.* 20:1324.  
 Busillo, J. M. et al. (2010) *J Biol Chem.* 285(10):7805.



Immunocytochemical labeling of CXCR4 in chick pluripotent cells. The cells were labeled with rabbit polyclonal CXCR4 (a.a. 328-338) antibody (CP4231), then detected using appropriate secondary antibody (Red). (Image provided by Dr. Yangqing Lu at the Regenerative Bioscience Center, University of Georgia).



Western blot analysis of human Jurkat cells treated with 100 nM calyculin A for 30 min. then the blots were untreated (-) or treated (+) with lambda phosphatase. The blots were probed with rabbit polyclonal anti-CXCR4 (Ser-324/Ser-325) (left panel), or anti-CXCR4 (a.a. 328-338) (right panel).

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**Immunogen****Uniprot ID: P61073**

CXCR4 (a.a. 328-338) synthetic peptide (coupled to carrier protein) corresponds to amino acids in the C-terminal region of human CXCR4. This sequence is conserved in mouse and rat CXCR4, but has low homology to other CXCR family members.

**Buffer and Storage**

Rabbit polyclonal, affinity-purified antibody is supplied in 100µl phosphate-buffered saline, 1 mg/ml BSA, and 0.05% sodium azide. Store at 4°C, stable for 6 months. For long term storage, aliquot and store at -20°C.

**Applications**

WB	1:250
ELISA	1:2000
ICC	1:100

**Species Reactivity**

Hu, Rt, Ms

End user should determine optimal dilution for their particular applications and experiments.

Western blot membranes were incubated with diluted antibody in 5% non-fat milk, Tris buffer, 0.04% Tween20 for 1 hour at room temperature.

Abbreviations: E = ELISA, ICC = immunocytochemistry, IHC = immunohistochemistry, IP = immunoprecipitation, MS = mass spectrometry, WB = western blot  
Hu = Human, Ms = Mouse, Rt = Rat, Ck = Chicken, F = Frog, B = Bovine

**Specificity**

The antibody detects bands at 35, 42, 50, and 100 kDa\* corresponding to various post-translationally modified forms of CXCR4 on SDS-PAGE immunoblots of human Jurkat and PC3 cells. These bands co-migrate with similar bands detected with anti-CXCR4 (Ser-324/Ser-325) and anti-CXCR4 (a.a. 322-329) antibodies.

\*All molecular weights (MW) are confirmed by comparison to MW standards and to western blot mobilities of known proteins with similar MW.

"Native" western blot utilizes non-reducing sample buffer (no mercaptoethanol or SDS), normal SDS-PAGE gel electrophoresis, and no methanol in transfer buffers.

**Related Products**

CK6500 CXCR4 Phospho-Regulation Antibody Sampler Kit

CP4251 CXCR4 (Ser-324/Ser-325), phospho-specific Rabbit Polyclonal

CP4211 CXCR4 (a.a. 322-329) Rabbit Polyclonal

CX4215 CXCR4 (a.a. 322-329) Blocking Peptide

CX4255 phospho-CXCR4 (Ser-324/Ser-325) Blocking Peptide

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