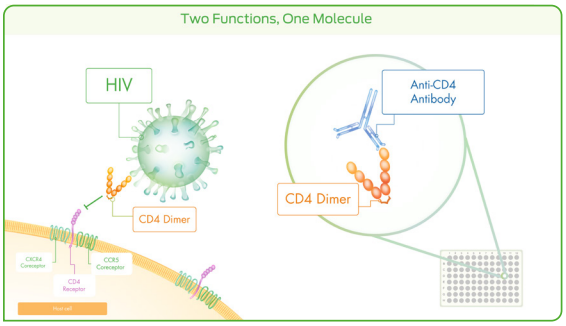
Application Note

# Enhanced HIV-1 Neutralization and Anti-CD4 Antibody Detection Using a Soluble CD4 Dimer

## Introduction

CD4 is a cell-surface glycoprotein expressed primarily on T helper lymphocytes and plays a central role in adaptive immune regulation. Beyond its physiological function, CD4 serves as the primary receptor for HIV-1 entry through high-affinity interactions with the viral envelope glycoprotein gp120. Engagement of CD4 induces conformational changes in gp120 that are required for subsequence co-receptor binding and viral fusion, making CD4 binding an obligate and defining step in HIV-1 infection (1). In addition to its central role in viral entry, CD4 can also become a target of the host immune response. Chronic HIV-1 infection has been associated with the development of anti-CD4 autoantibodies in a subset of individuals, although their prevalence, specificity, and functional consequences remain under active investigation (2, 3). Reliable detection and characterization of these autoantibodies requires CD4 antigens that faithfully preserve native structure and biologically relevant epitopes.




For recombinant CD4 intended for autoantibody screening, structural integrity is essential for assay validity. Many commercially available reagents are monomeric constructs that may not fully recapitulate the spatial orientation, stability, or epitope accessibility of native CD4. Because CD4-dependent interactions are inherently conformational, even subtle differences in folding or valency can alter antibody binding profiles. Insufficiently validated antigens risk underrepresenting clinically relevant autoantibody populations or generating artifacts due to non-native epitope exposure. Functional validation is therefore essential to confirm that a recombinant CD4 reagent maintains structural integrity consistent with its biological role.

To address these challenges, we developed a dimeric CD4 reagent designed to more closely reflect the multivalent and conformational context of the native receptor. In this application note, we present functional validation data alongside screening performance results, demonstrating the suitability of the dimeric CD4 reagent for sensitive and biologically relevant detection of anti-CD4 autoantibodies.

## Results

**Recombinant Dimeric CD4 Preserves Native Epitope Presentation and gp120-Specific Binding.**

To confirm the biochemical integrity of the recombinant CD4 dimer, protein purity was first assessed by SDS-PAGE under non-reducing conditions. A single predominant band corresponding to the expected molecular weight of the dimeric construct was observed with no detectable lower molecular weight species or aggregation products (Figure 1A). These data indicate high purity and structural homogeneity of the recombinant protein.

We next evaluated whether the dimer preserves native CD4 epitopes using an ELISA-based binding assay with a Keliximab (Figure 1B). The recombinant CD4 dimer demonstrated specific and potent binding to the antibody, with an EC50 of 6.8 ng/mL. This high affinity interaction confirms that the construct maintains conformational integrity and properly presents native antibody-recognized epitopes.

To further assess functional ligand recognition, binding to HIV-1 gp120 was examined and compared to binding to the gp120 D368R mutant, which disrupts the canonical CD4 binding site (Figure 1C) (1). The CD4 dimer exhibited robust binding to wild-type gp120, whereas binding to the D368R mutant was diminished. The loss of interaction with the D368R variant confirms that binding is specific to the native CD4-binding interface on gp120. Collectively, these results demonstrate that Conigen's high quality dimeric CD4 preserves conformational epitopes recognized by anti-CD4 antibodies, and engages gp120 through the expected CD4-binding site.

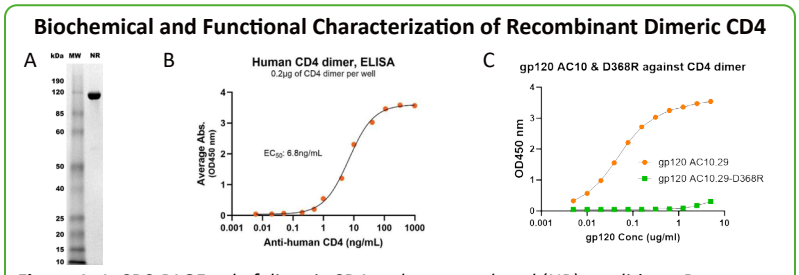
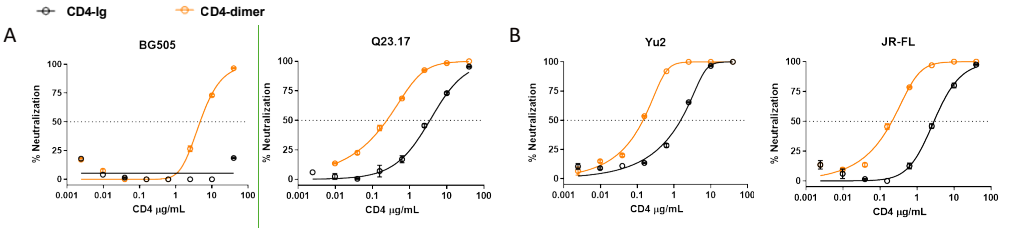



***Figure 1.*** *A. SDS-PAGE gel of dimeric CD4 under non-reduced (NR) conditions. B. Immobilized human CD4 dimer protein can bind anti-human CD4 monoclonal antibody with half maximal effective concentration (EC50) range of 3.46 to 13.85 ng/mL. C. CD4 dimer binds to gp120 AC10.29 (quantify) and D368R diminishes binding.*

**Dimeric CD4 Exhibits Enhanced HIV-1 Neutralization Potency Compared to Monomeric CD4.**

To functionally evaluate whether our recombinant CD4 construct preserves the native spatial orientation and gp120-binding competence of cellular CD4, we performed a pseudovirus neutralization assay. Pseudoviruses were pre-incubated with serial dilutions of soluble proteins (CD4-Ig monomer and Conigen's CD4 dimer) and then added to TZM-bl cells, widely used for quantitative HIV infection assays. Infection levels were quantified by luciferase reporter activity, providing a direct measure of viral entry.

**Conigen's CD4 Dimer Displays Broad Neutralizing Activities Against HIV-1 Tier 2 Viruses**

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| HIV-1 Subtype | $IC_{50}$ (ug/mL) | |
|---|---|---|
| | CD4 Dimer | CD4-Ig |
| Q23.17 | 0.24 | 3.52 |
| BG505 | 4.73 | >40 |
| Yu2 | 0.14 | 1.52 |
| JR-FL | 0.21 | 2.88 |

***Figure 2.*** *A. Neutralizing assays against HIV-1 tier 2 subtype viruses Q23.17 and BG505. B. Neutralization assays against HIV-1 tier 2 subtype B viruses Yu2 and JR-FL. C. $IC_{50}$ (ug/mL) values for HIV-1 neutralization assays shown in A and B.*

Our engineered dimeric CD4 demonstrated markedly enhanced neutralization activity compared to monomeric CD4-Ig for both HIV-1 tier 2 subtype A (Figure 2A) and subtype B (Figure 2B). Specifically the dimer exhibited approximately a 10-fold increase in neutralization potency, as measured by IC50 values (Figure 2C). While CD4-Ig reduced infection in a dose-dependent manner, the CD4 dimer achieved equivalent inhibition at substantially lower concentrations. This increased potency of the dimeric CD4 suggests improved functional avidity for gp120, consistent with preserved epitope accessibility and favorable spatial orientation of the CD4 binding domains. These results inculcate that the recombinant dimer recapitulates key structural and functional features of native CD4 and engages gp120 in a biologically relevant manner.

**Dimeric CD4 Enables Detection of Anti-CD4 Autoantibodies in HIV-1 Patient Samples.**

Anti-CD4 autoantibodies have been reported in a subset of individuals with chronic HIV-1 infection, particularly in the setting of advanced disease and sustained immune activation (2, 3). To evaluate whether our recombinant CD4 dimer could serve as an improved tool for detecting anti-CD4 antibodies, we screened purified IgG isolated from plasma samples of HIV-1 infected individuals using an ELISA-based format. For comparison, a commerciallyavailable soluble CD4 monomer (Company X) was used under identical assay conditions.

When monomeric CD4 was used as the capture antigen, no detectable anti-CD4 reactivity was observed across the patient samples tested. In contrast, screening with Conigen's dimeric CD4 construct revealed measurable anti-CD4 antibody binding in two patient samples. The observed reactivity was reproducible and above background thresholds established using healthy donor controls. The differential detection between monomeric and dimeric CD4 indicates that antigen configuration strongly influences autoantibody recognition, with dimeric CD4 likely improving epitope presentation, structural stability, and avidity.

**Conigen's CD4 Dimer Detects Anti-CD4 Autoantibodies in Patient Samples**

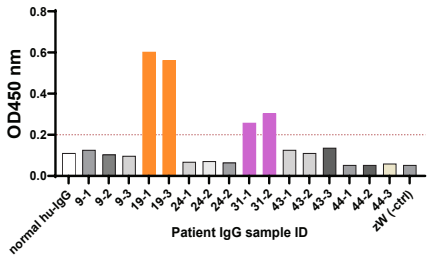


***Figure 3.*** *Purified IgG isolated from plasma samples of HIV-1 infected individuals (n=16) was screened using an ELISA-based format.*

## Conclusion

Reliable detection of anti-CD4 autoantibodies depends on reagents that preserve the native structure and functional binding properties of CD4. Conigen's recombinant dimeric CD4 maintains high purity, preserves conformational epitopes recognized by anti-CD4 antibodies.

Notably, the dimeric reagent detected anti-CD4 autoantibodies in HIV-1 patient samples that were missed using a conventional monomeric CD4 reagent under the same conditions, highlighting the importance of antigen conformation and suggesting monomeric reagents may underestimate these antibodies.

**References**


1. Kwong PD, et al, *Nature* 1998, 393:648
2. Epling B, et al., *Clin Infect Dis* 2025, 80:1340
3. Luo Z, et al., *J Virol* 2021, 95:e00271-21