Datasheet

CD20, Active Protein
(Recombinant Human Cluster of Differentiation 20)

Catalog Number: **41-R501A-10UG, 41R501A-50UG, 41-R501A-BULK**

Contents: 10 µg, 50 µg or Bulk quantities of protein

Background

CD20 is a transmembrane receptor, specific of normal and malignant B-cells. Indeed, CD20 is involved in development and differentiation of B-cells. It boosts calcium signal indirectly and regulates their cellular signalization. CD20 can be subjected by post-translational modifications (phosphorylation). Moreover, it can be found as a double-barrel dimer. As of today, there is no natural ligand known and its function is still debated. CD20 transmembrane receptor is targeted in various diseases like cancer (non-Hodgkin's lymphoma and Chronic lymphocytic leukemia's), multiple sclerosis, or autoimmune disorders. The treatment most used for lymphoid malignancies is the Rituximab, a monoclonal antibody, which induced the clustering of CD20 in lipid raft. Thus, this triggers several immune defense mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP).

Protein Information

Target Name:	Cluster Differenciation 20 (CD20)
Accession Number:	P11836 (UniProt)
Class:	Transmembrane receptor
Sequence:	Full-length, wildtype sequence, with a C-terminus Avi Tag HRV3C protease cleavage site, TwinStrep-Tag and a 10xHis-tag MTTPRNSVNGTFPAEPMKGPIAMQSGPKPLFRRMSSLVGPTQSFFMRESKTL GAVQIMNGLFHIALGGLLMIPAGIYAPICVTVWYPLWGGIMYIISGSLLAATEKN SRKCLVKGKMIMNSLSLFAAISGMILSIMDILNIKISHFLKMESLNFIRAHTPYINIY NCEPANPSEKNSPSTQYCYSIQSLFLGILSVMLIFAFFQELVIAGIVENEWKRT CSRPKSNIVLLSAEEKKEQTIEIKEEVVGLTETSSQPKNEEDIEIIPIQEEEEEE TETNFPEPPQDQESSPIENDSSPGGGLNDIFEAQKIEWHEGGGLEVLFQGP GGGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEKGGGHHHHHHHHHH
Affinity Tag:	Avi/TwinStrep/His (both C-terminal)
Origin:	Human (Homo sapiens)
Theoretical Molecular Weight:	40.8 kDa
Expression System:	HEK 293F cells
Sample Buffer:	50 mM Tris, 150mM NaCl, 10% glycerol, 0.03%-0.003% DDM-CHS

Shipping and Storage Information

Shipping: Dry ice

Storage: Store at -80°C, avoid freeze-thaw cycle

Quality Controls

Purity

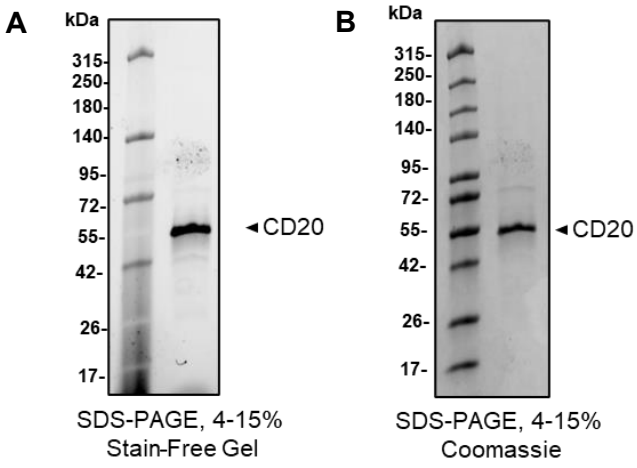

Figure 1: SDS-PAGE, Stain-Free™ detection (left) and Western Blot (right) of CD20 receptor.
(**A**) Purified CD20 was migrated on a 4-15% Tris-glycine SDS-PAGE. (**B**) Total proteins were detected using Stain-Free™ detection then stained with InstantBlue™ Coomassie. Black arrow indicates full-length CD20 with a purity of 95%.

Cryostability

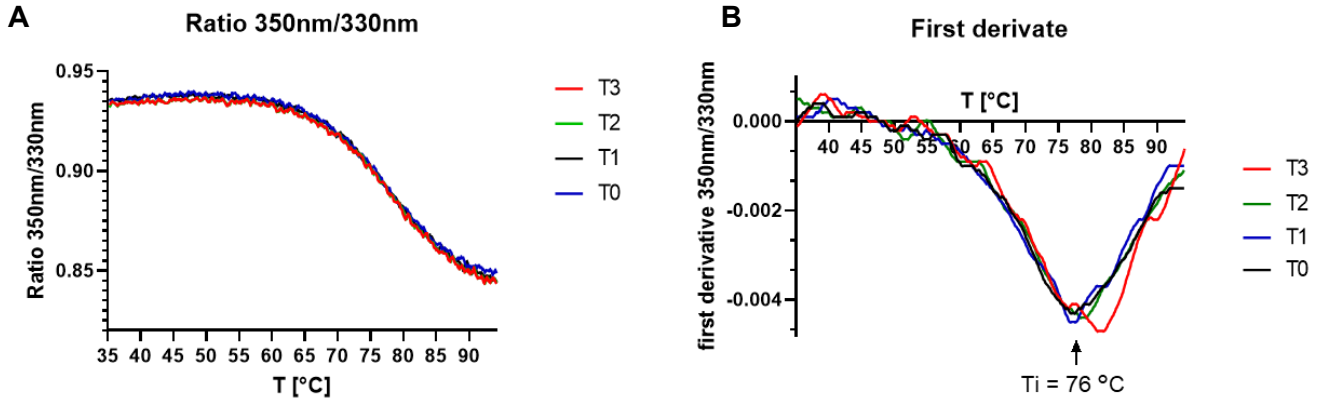

Figure 2: Thermal shift assays (TSA) and first derivative profiles measured as the ratio between profiles brightness at 350 nm and 330 nm for the CD20 receptor.
Cryostability was measured to test the possibility of storing the protein at -80°C, and to see the behavior over several freeze-thaw cycles. (**A**) Cryostability analysis is performed by rapid TSA (raw data ratio 350 nm/330 nm) and (**B**) analysis of changes in fluorescence emission properties of tryptophan residues on a NanoTemper Tycho device with first derivative data ratio 350 nm/330 nm. The resulting unfolding profiles are analyzed for the inflection points (Ti, shift or loss indicate destabilization or unfolding), initial ratios (increase indicates denaturated protein), Δ ratios (decrease indicates the presence of more unfolded protein) and sample brightness (decrease indicates loss of protein). CD20 shows an inflection temperature of 76 °C (indicated by an arrow), Ti is not affected by the three freeze-thaw cycles.

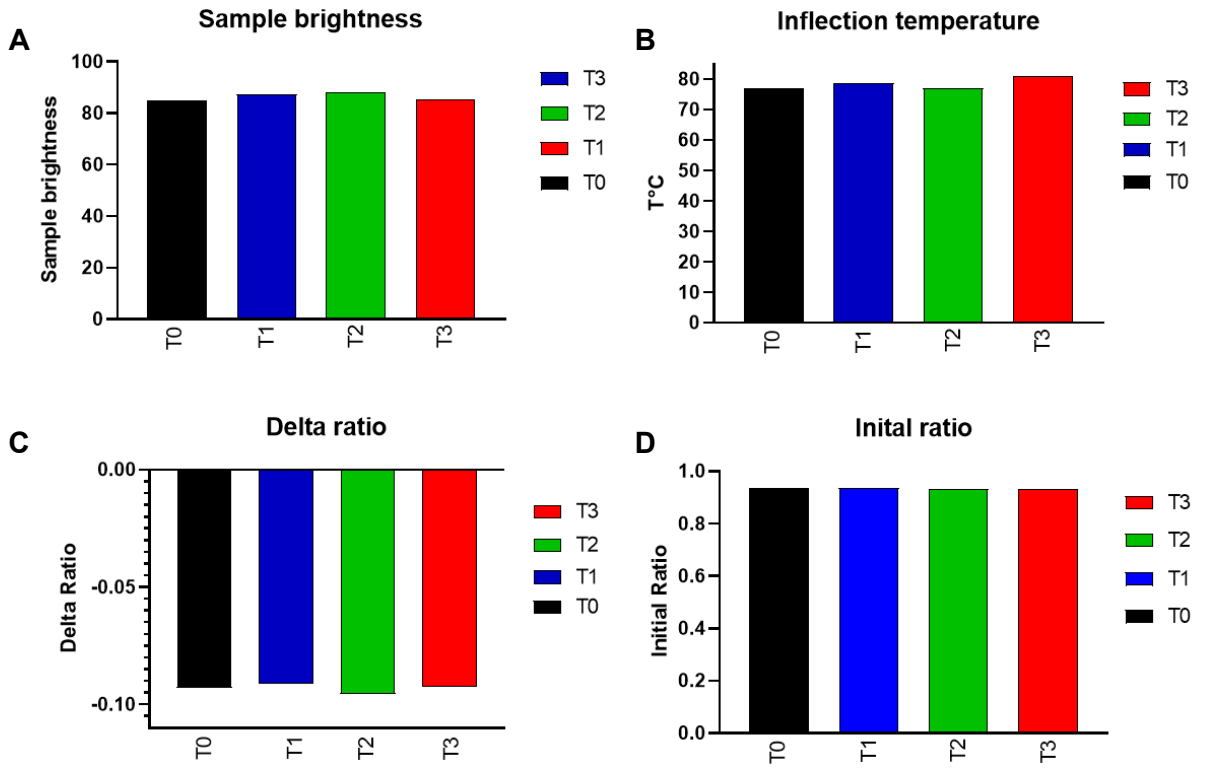

Figure 3: Summary of the key parameters of the cryostability of CD20 receptor.
(**A**) Sample brightness values, (**B**) inflection temperatures, (**C**) delta-ratio values, and (**D**) initial ratio values measured during the cryostability using NanoTemper Tycho device. All the key parameters remain stable after 3 freeze-thaw cycles, showing CD20 freeze-thaw cycles does not lead to protein aggregation, unfolding or loss of protein.

Ligand Binding Assay

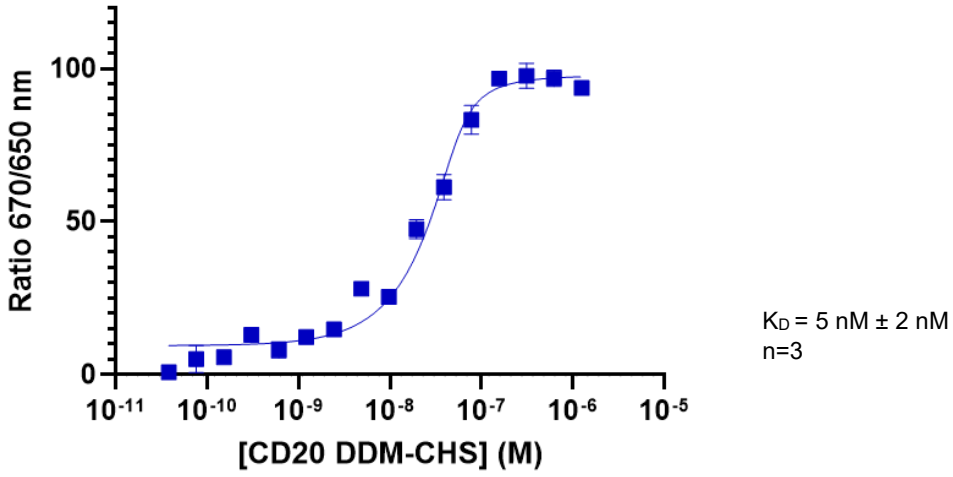

Figure 5: CD20 – Rituximab binding assay measured by spectral shift.
Binding affinity of monoclonal antibody Rituximab was measured by following the changing in fluorescence in spectral shift using a Monolith X (NanoTemper) device. Rituximab was labeled using a Red-NHS dye that carries a reactive NHS-ester group that reacts with primary amines (lysine residues) to form covalent bond. A final concentration of 50 nM Rituximab labeled with RED-NHS was incubated with a range of CD20 concentration. The detection of spectral shift is achieved through dual wavelength detection at 650 nm and 670 nm, and the K_D is then calculating by plotting the ratios 670 nm/650 nm against the concentration of CD20. The experiment was performed in triplicate. A K_D of 5 nM was measured demonstrating CD20 biotinylated is in active conformation.

References

- Agez M et al. Biochemical and biophysical characterization of purified native CD20 alone and in complex with rituximab and obinutuzumab. *Scientific report.* 2019 August. DOI: 10.1038/s41598-019-50031-4

Additional Information

Restricted Use: Limited Use Label License

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Limited Product Warranty

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