Recombinant Human Z-UBQLN2-7 scFv Antibody

Gene Name	UBQLN2
Protein Name	Ubiquilin 2
Synonyms	ALS15, CHAP1, DSK2, HRIHFB2157, N4BP4, PLIC2
Uniprot ID	Q9UHD9
Description	Plays an important role in regulation of different protein degradation mechanisms and pathways including ubiquitin-proteasome system (UPS), autophagy, and endoplasmic reticulum-associated protein degradation (ERAD) pathway. Mediates proteasomal targeting of misfolded or accumulated proteins for degradation by binding (via UBA domain) to their polyubiquitin chains and interacting (via ubiquitin-like domain) with the subunits of the proteasome [1]. Plays a role in the ERAD pathway via its interaction with ER-localized proteins FAF2/UBXD8 and HERPUD1 and may form a link between polyubiquitinated ERAD substrates and the proteasome [2, 3]. Involved in regulation of macroautophagy and autophagosome formation; required for maturation of autophagy-related protein LC3 from the cytosolic form LC3-I to the membrane-bound form LC3-II and may assist in the maturation of autophagosomes to autolysosomes by mediating autophagosome-lysosome fusion [4, 5]. Negatively regulates the endocytosis of GPCR receptors: AVPR2 and ADRB2, by reducing the rate at which receptor-arrestin complexes concentrate in clathrin-coated pits (CCPs) [6]. UBQLN2 has been implicated in amyotrophic lateral sclerosis (ALS) [7, 8].
Antigen Coverage	Full-length construct, 624 aa
Antigen Sequence (Antigen - C-terminal linker and Avi tag)	SMAENGESSGPPRPSRGPAAAQGSAAAPAEPKIIKVTVKTPK EKEEFAVPENSSVQQFKEAISKRFKSQTDQLVLIFAGKILKDQ DTLIQHGIHDGLTVHLVIKSQNRPQGQSTQPSNAAGTNTTSAS TPRSNSTPISTNSNPFGLGSLGGLAGLSSLGLSSTNFSELQSQ MQQQLMASPEMMIQIMENPFVQSMLSNPDLMRQLIMANPQM QQLIQRNPEISHLLNNPDIMRQTLEIARNPAMMQEMMRNQDL ALSNLESIPGGYNALRRMYTDIQEPMLNAAQEQFGGNPFASV GSSSSSGEGTQPSRTENRDPLPNPWAPPPATQSSATTSTTT STGSGSGNSSSNATGNTVAAANYVASIFSTPGMQSLLQQITE NPQLIQNMLSAPYMRSMMQSLSQNPDLAAQMMLNSPLFTAN PQLQEQMRPQLPAFLQQMQNPDTLSAMSNPRAMQALMQIQ QGLQTLATEAPGLIPSFTPGVGVGVLGTAIGPVGPVTPIGPIGP IVPFTPIGPIGPIGPTGPAAPPGSTGSGGPTGPTVSSAAPSETT SPTSESGPNQQFIQQMVQALAGANAPQLPNPEVRFQQQLEQ LNAMGFLNREANLQALIATGGDINAAIERLLGSQPSSSKGGYG LNDIFEAQKIEWHE
Z-UBQLN2-7 scFv Sequence (V_H - linker - V_L)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCARTYYGDTMDYWGQGTLVTVSSGGGGS GGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSY LNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQPGVYYLPTFGQGTKLEIK

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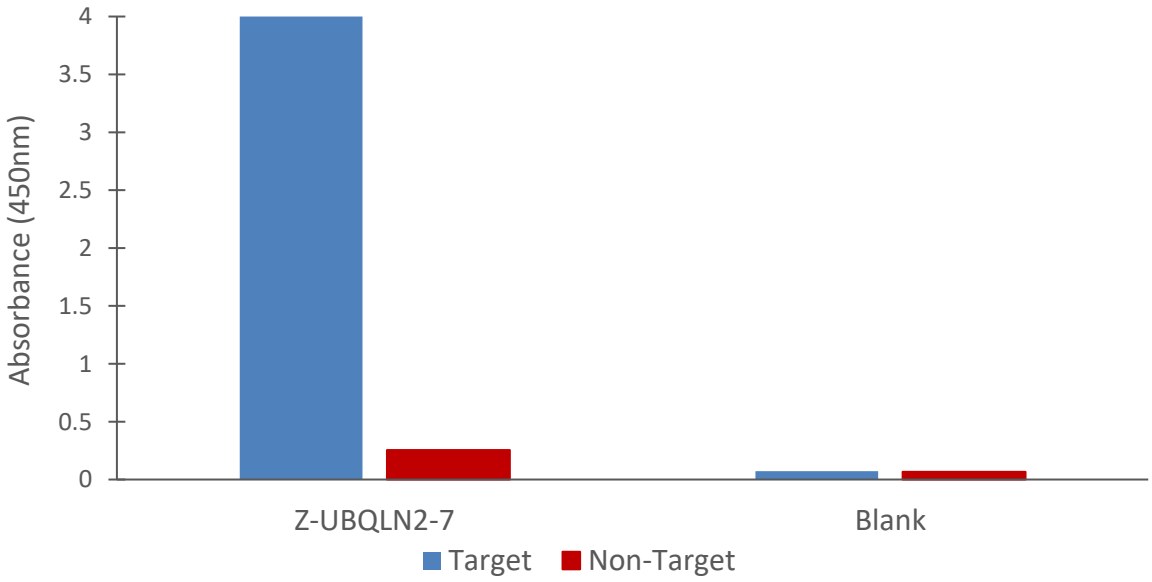
Antibody Fragment Generation and Production	Z-UBQLN2-7 was generated by phage display technology from a human synthetic scFv library. The scFv was produced by bacterial expression in *E. coli,* with secretion to the periplasm, and purified by protein A affinity. The final protein batch of Z-UBQLN2-7 scFv also carries a C-terminal His_6 tag and a 3xFLAG tag.
Validated Applications	ELISA, HTRF, Luminex, SPR, and IP-MS.

Enzyme-Linked ImmunoSorbent Assay (ELISA):

Method Description	ELISA screens were performed to assess binding of selected single chain fragment variable (scFv) antibody clones. Each clone was tested in duplicate against its target antigen and an unrelated non-target control (Primary ELISA). Positive clones were selected and screened against its target antigen and a streptavidin control (Secondary ELISA). ELISA screens were performed in 384 well plates coated with [1ug/mL] streptavidin. Biotinylated antigens were diluted in PBT buffer (0.5% BSA, 0.05% Tween 20 in PBS) to [1ug/mL] and added prior to the addition of scFv bacterial culture supernatants. Bound scFvs, which contain a FLAG-tag, were detected using an HRP conjugated anti-FLAG M2 antibody (Sigma, [1:10,000]). TMB substrate solution (Thermo Scientific) was used as a chromogenic substrate, before the final addition of 1M H_2SO_4 stop solution. Plates were washed four times in between each step listed above with PBS-T (0.05% Tween 20 in PBS). Absorbance was measured at 450nm. Only scFv clones with high enough absorbance ratios (Target / Non-Target and Target / Streptavidin) were selected for further analysis. ELISA results for Z-UBQLN2-7 are shown below (blank indicates no scFv was added).

Z-UBQLN2-7 binding in Primary ELISA screen:



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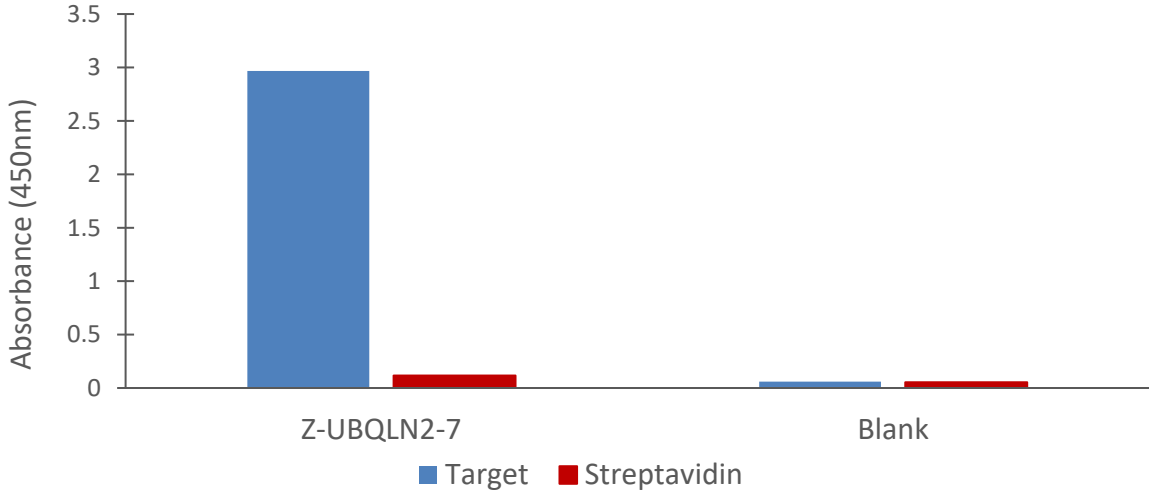
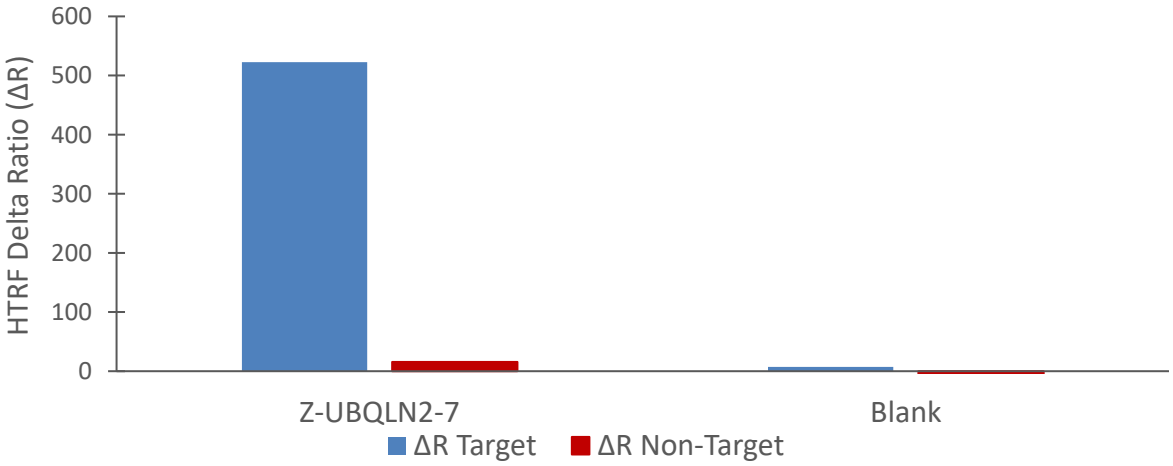
Z-UBQLN2-7 binding in Secondary ELISA screen:



Homogenous Time Resolved Fluorescence (HTRF):

Method Description	HTRF technology was employed to robustly and reliably determine, in solution, the binding of each scFv antibody to its target antigen. Sequence unique clones, that tested positive in ELISA, were evaluated by HTRF. Supernatants from bacterial cultures containing scFvs were diluted in assay buffer (0.1% BSA in PBS), then mixed with biotinylated target antigens to a final concentration of 50 nM. Donor anti-FLAG M2 antibody labeled with Terbium (Cisbio) and acceptor Streptavidin labeled with XL665 (Cisbio) were added to each scFv in 384 well plates. Samples were incubated for 2 hours at RT. The binding signal (665nm) and the background/noise signal (615nm) were measured using a EnVision instrument (PerkinElmer). HTRF ratios (665nm Acceptor / 620nm Donor) were calculated. Delta ratios (ΔR) were determined by subtracting the background from the mean emission fluorescence ratio for each sample. Delta ratios were plotted to compare the energy transfer signal for each scFv to its non-target control. Results for Z-UBQLN2-7 are shown in the graph below (blank indicates no scFv was added).

HTRF results for Z-UBQLN2-7:



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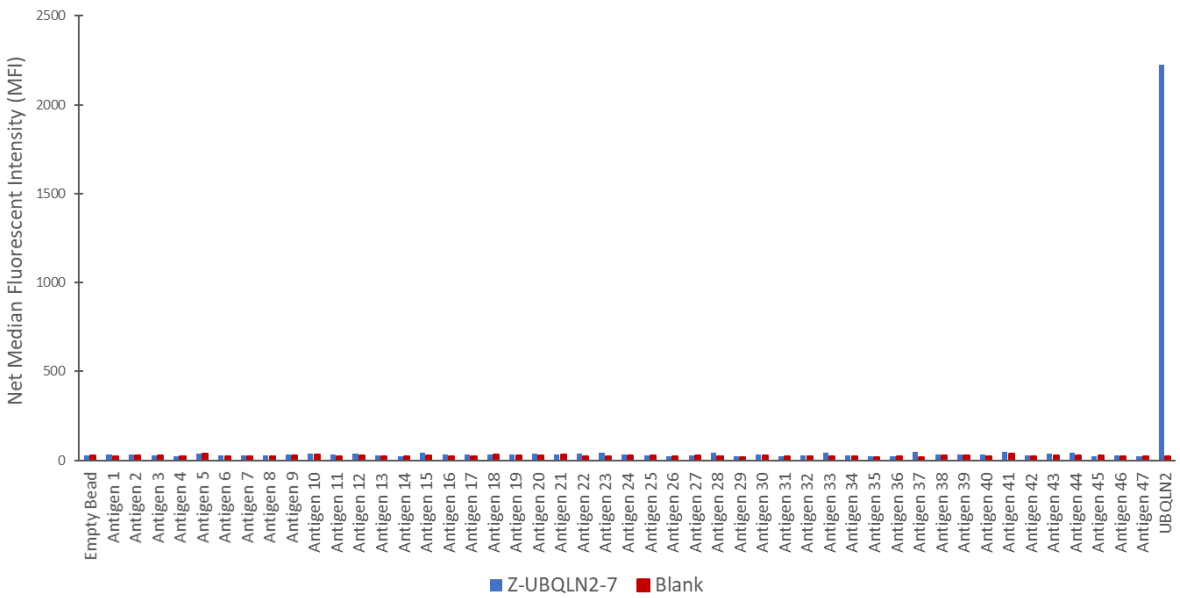
Luminex Assay:

Method Description	The specificity of the anti-UBQLN2 scFv antibodies were evaluated against 48 different antigens (including UBQLN2) using a bead based multiplex Luminex assay. Color-coded magnetic beads (Magplex Luminex Corp.) were activated with [5mg/mL] NHS and EDC in activation buffer (0.1M NaH_2PO_4, pH 6.2), then added to 96 well plates and incubated with shake at RT for 20min. Beads were washed twice with MES buffer (0.05M 2-(N-morpholino) ethane sulfonic acid, pH 5.0) before adding neutravidin and incubating for 2 hours at RT with shake. Beads were then washed twice with PBS-T before storage buffer (Blocking Reagent for ELISA (Roche) with 0.1% ProClin300 (Sigma) was added and incubated overnight at 4°C. Storage buffer was removed and biotinylated proteins (1μM in PBS) were added to neutravidin coupled beads then incubated for 1 hour at RT with shake. Plates were washed 3x with PBS-T, storage buffer was added, and incubated at 4°C overnight. scFv antibodies were diluted in assay buffer (3% BSA, 0.05% Tween 20, and [10μL/mL] neutravidin in PBS) and incubated for 1 hour at 4°C. A beadstock was prepared with coupled beads in storage buffer at a concentration of 100beads/ID/μL. The beadstock solution (5μLs) was mixed with each scFv (45μL) in 384 well plates and incubated for 1 hour at RT with shake. Plates were washed 3x with PBS-T and anti-FLAG RPE (Prozyme, [1:1000]) was added for 30min at RT with shake. Plates were washed 3x in PBS-T and samples were analyzed on a FLEXMAP3D (Luminex Corp). Luminex results for Z-UBQLN2-7 are plotted below. Graph illustrates the degree of specificity of Z-UBQLN2-7 to bind to its respective antigen (UBQLN2) and none of the other 47 antigens (blank indicates no scFv was added).

Luminex results for Z-UBQLN2-7:



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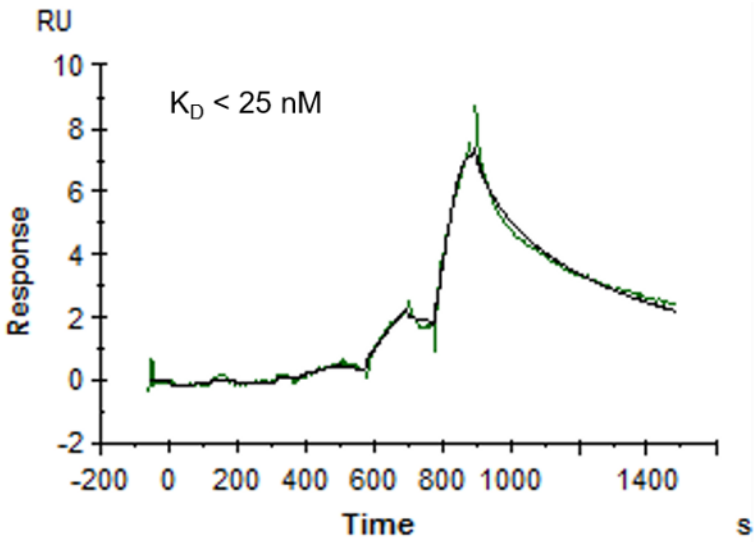
Surface Plasmon Resonance (SPR) Affinity Measurements:

Method Description	Kinetic measurements were performed using SPR on a Biacore T200 (GE Healthcare). The anti-FLAG M2 antibody (Sigma) was used to capture the FLAG-tagged scFv before antigen injection. Single Cycle Kinetics was performed. Accordingly, five concentrations of antigen are sequentially injected in increasing order within the same cycle before regeneration with Glycine-HCl, pH 2.5. Antigen concentrations tested against Z-UBQLN2-7 include: 0.32, 1.6, 8, 40, and 200nM with a flowrate of 30µL/min. The kinetic constants were calculated using the Biacore T200 Evaluation Software 3.1 and the 1:1 Langmuir binding model, after removal of the reference cycle (running buffer instead of antigen) and the reference channel. Transient spikes from *e.g.* air bubbles were also removed from the obtained sensorgrams. The Single Cycle Kinetic sensorgram for Z-UBQLN2-7 is shown below.

Single Cycle Kinetics curve for Z-UBQLN2-7:



Preliminary data - optimization on-going

Immunoprecipitation – Mass Spectrometry (IP-MS):

Method Description	Immunoprecipitation followed by mass spectrometry was employed to verify the interaction specificity of each scFv antibody with its intended target. Immunoprecipitation was performed on HEK293 cell lysates expressing endogenous Ubiquilin 2 protein as previously described [9]. Obtained samples were loaded onto a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific) coupled to an o-QTOF impact II™ (Bruker Daltonics) mass spectrometer with a Captive Spray ion source (Bruker Daltonics). Data was analyzed with Protein Scape software (Bruker Daltonics) using Mascot search engine (Matrix Science Ltd.). Normalized spectral abundance factor (NSAF) values were calculated as previously described [9].

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Z-UBQLN2-7 was able to capture endogenous Ubiquilin 2 from HEK293 cell lysates. The target protein was the first specific hit in the list of immunoprecipitated proteins with an NSAF value of 22 and 4 unique peptides detected. No other specific interactors were found. More detailed data can be shared upon request.

Comments and Contact:

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References:

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