p62 ELISA kit

Highly validated, quantitative p62 ELISA kit available for autophagy research.

The p62 ELISA kit is a colorimetric, immunometric immunoassay kit with results in 3 hours.

The generic term ‘‘autophagy’’ comprises several processes by which the lysosome acquires cytosolic cargo, with three types of autophagy being discerned in the literature: (1) macroautophagy, characterized by the formation of a crescent-shaped structure (the phagophore) that expands to form the double-membrane autophagosome, capable of fusion with the lysosome; (2) microautophagy, in which lysosomes invaginate and directly sequester cytosolic components; and (3) chaperone-mediated autophagy (CMA), which involves translocation of unfolded proteins across the lysosomal membrane.

Upregulation of autophagy pathways occurs in response to extra- or intracellular stress and signals such as starvation, growth factor deprivation, ER stress and pathogen infection. Malfunction of these pathways is linked to various human pathologies including cancer, neurodegeneration and infectious diseases.

Selective macroautophagy describes the pathway of self-degradation of whole cellular components, protein aggregates or unusually long-lived proteins; in which double-membrane autophagosomes sequester organelles, ubiquitinylated proteins or ubiquitinylated protein aggregates and subsequently fuse with lysosomes for breakdown by resident hydrolases. Autophagic clearance of protein aggregates requires the ubiquitin-binding receptors p62 and NBR1.

The p62 protein, also known as sequestosome 1 (SQSTM1), has a dual functionality as both a scaffold protein and aiding in trafficking for protein degradation. It can polymerize and bind to NBR-1 via a PB1 (Phox and Bem1) domain, interact with ubiquitinated proteins linking them to the autophagic machinery via a UBA (ubiquitin-associated) domain and bind to the LC3II protein of the autophagy pathway through an LIR (LC3 interacting region) motif. p62 provides a key link between the ubiquitin-proteasome system (UPS) and autophagy by facilitating autophagic degradation of ubiqutinated proteins, decreasing aggregation of misfolded and non-functional proteins within cells, resulting in enhanced cellular survival characteristics. Because p62 accumulates when autophagy is inhibited, and decreased levels can be observed when autophagy is induced, p62 may be used as a biomarker to study autophagic flux. Also, p62 has been implemented in neurodegenerative diseases such as Parkinson's and

- Highly sensitive assay, measure as little as 100 pg/ml of p62
- Fully quantitative results surpass semi-quantitative Western blot analysis
- Higher throughput format, assay up to 40 samples in duplicate in just 3 hours
- Easy-to-use liquid color-coded reagents reduce errors

Alzheimer's and in the skeletal disorder Paget's disease of bone, establishing the p62 protein as a potential therapeutic target.

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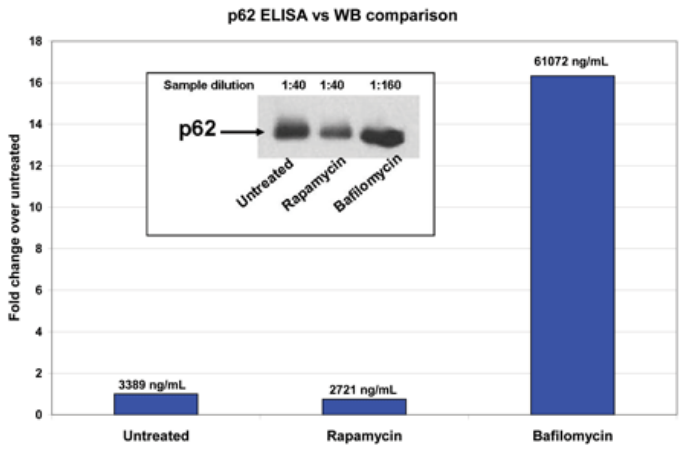
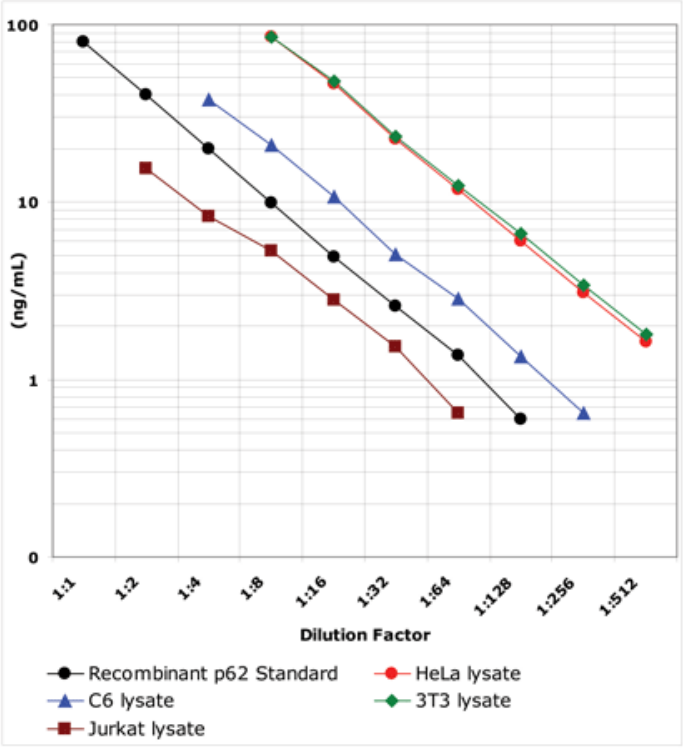
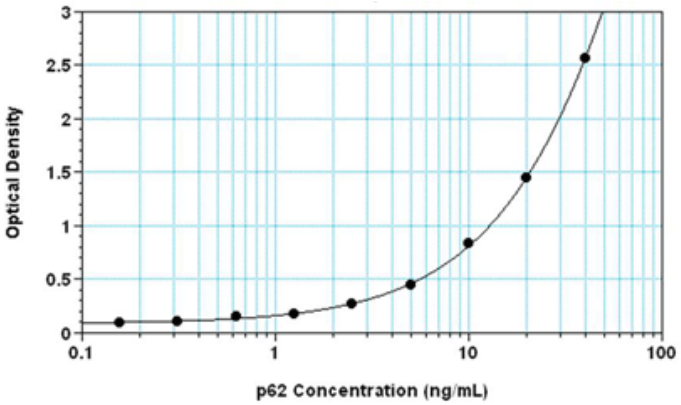
ADI-900-212-0001	96 wells

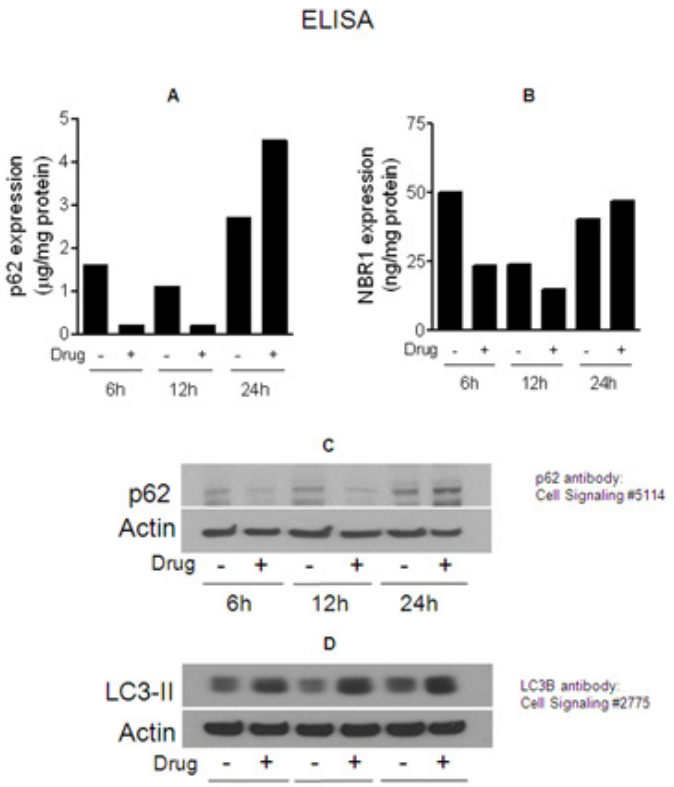
Manuals, SDS & CofA View Online »



Stimulation Assay, p62 ELISA vs WB comparison: Human HeLa cells were treated for 24 hours with 800nM Rapamycin, an inducer of autophagy, or 800nM Bafilomycin A1, an inhibitor of vacuolar ATPase which leads to an accumulation of autophagosomal structures. Cells were then harvested, washed and lysed at 5×10^6 per mL RIPA Cell Lysis Buffer 2 following the procedure in the Cell Lysate Preparation section. These samples were diluted and resolved on an 80-12% Tris-glycine gel, transferred to nitrocellulose membrane and probed for p62. The same cell lysates were also diluted in assay buffer and run in this kit. The Western Blot provided a visual for both the decrease and increase in p62 levels after treatment of cells in culture. The p62 ELISA kit was able to assign values to the amounts of p62 protein (numbers above each bar) thus allowing a quantitative comparison of the decrease with Rapamycin treatment (~25%) and the increase with Bafilomycin A1 treatment (~1600%).







Correlation of p62 (Prod. No. ADI-900-212) and NBR1 (Prod. No. ADI-900-211) immunoassays to autophagy induction. MDA-MB-231 human breast cancer cells were treated with 2µM of withaferin A (WA), an autophagy inducing drug. Cells were harvested at 6, 12 and 24 hours post-treatment and lysed in RIPA cell lysis buffer 2 containing protease inhibitors and DNase. Cell lysates were clarified by centrifugation and analyzed in p62 assay (Figure 3A) and NBR1 assay (Figure 3B). Concentration of antigen was normalized to total cellular protein. Cell lysates were resolved by SDS-PAGE and analyzed by Western blot using anti-p62 antibody from Cell Signaling, CN #5114 (Figure 3C) or anti-LC3 antibody from Cell Signaling, CN #2775 (Figure 3D). Signal intensity in both Western blots were compared to actin levels and numbers above band represent changes in protein levels relative to corresponding DMSO-treated control.

Handling & Storage

Use/Stability	Store all components at +4ºC, except Standard at -80ºC.
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Sequestosome 1
Application	Colorimetric detection, ELISA
Application Notes	For the quantitative determination of human, rat and mouse p62 in cell lysate samples. Cited sample type includes in PBMC lysates.
Assay Time	3 hours
Compatibility	This product is compatible with the **Absorbance 96 Plate Reader**.
Contents	Microtiter Plate, Antibody, Conjugate, Assay Buffer 13, Wash Buffer Concentrate, Standard, TMB Substrate, Stop Solution 2, RIPA Cell Lysis Buffer 2
Crossreactivity	No cross reactivity.
Sensitivity	100pg/ml (range 625 – 40,000pg/ml)
Species Reactivity	Human, Mouse, Rat
UniProt ID	Q13501 (human)
Wavelength	450 nm

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