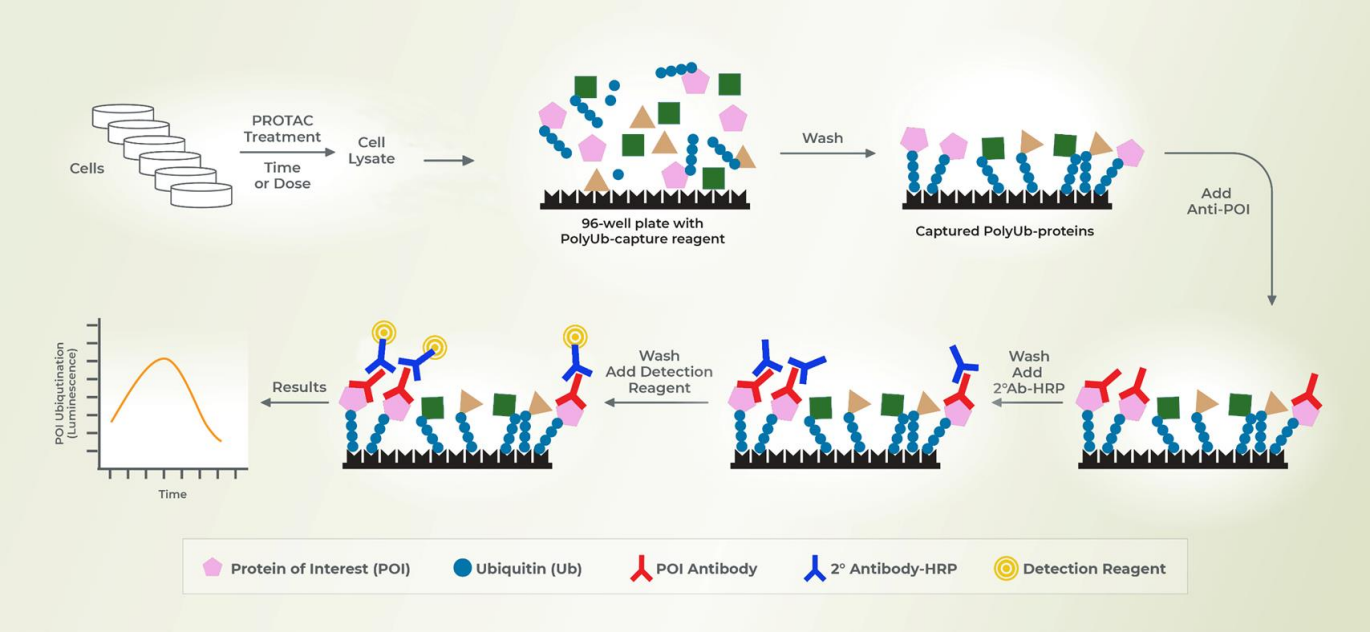
LifeSensors from genomics to proteomics

K48 Ubiquitin Linkage ELISA Kit
(Chain Selective)

Instruction Manual

Cat. No. PA480



Intended Use

To enrich and quantitate -K48 ubiquitination in cellular and tissue lysates.

The Ubiquitin Proteasome System (UPS) is a dynamic process involved in various cellular functions, including cell signaling, DNA repair, and proteasomal degradation. Ubiquitin can form diverse chains on its seven lysine residues, with K48-linked polyubiquitination being a crucial post-translational modification that drives protein degradation and maintains cellular homeostasis. Lifesensors' K48 Ubiquitin Linkage ELISA Kit enables relative and absolute quantitation of K48 ubiquitination on target proteins in cellular and tissue lysates under various treatments and conditions.

This assay replaces labor-intensive, semi-quantitative Western blot methods for examining K48 polyubiquitination and protein degradation, offering quantitative, reproducible results and facilitating high-throughput screening for compound library processing and structure-activity relationship (SAR) studies.

*The plate is designed for research use only.

Principle of the assay

The K48 Ubiquitin Linkage Assay Kit is a sandwich ELISA-based assay that captures K48 polyubiquitinated proteins from cell lysates in wells pre-coated with a proprietary K48 polyubiquitin capture reagent. Non-polyubiquitinated proteins or those ubiquitinated on lysine residues other than K48 are removed by washing. An antibody directed against the target protein is then used for quantitation. A secondary antibody conjugated to horseradish peroxidase (HRP) generates a measurable chemiluminescence signal on a microplate reader, which directly correlates with the levels of K48-linked polyubiquitinated target proteins.

Components

Coated plates:	One pre-coated and blocked 96-well strip plate (***Store at -80°C***)
Blocking concentrate (BC):	12 mL of 5X blocking agent for antibody dilution (***Store at 4°C***)
Plastic plate seals:	Two provided.
Detection reagent 1 (DR1):	1.0 mL vial of DR1 reagent (***Store at 4°C***)
Detection reagent 2 (DR2):	1.0 mL vial of DR2 reagent (***Store at 4°C***)
UPS Inhibitor cocktail (100X)	100 µL vial (in DMSO; ***Store at -20°C***)

(Contains protease inhibitor cocktail, PMSF, PR-619, MG-132 and 1,10-phenanthroline)

Components required but not supplied

PBS and PBS containing 0.1% Tween 20 (PBS-T) or TBS / TBS-T for phosphorylated targets.

Phosphatase Inhibitors as needed for studying phosphorylated targets.

Disposable gloves, pipette tips, reagent reservoirs

Multi-channel pipettor, Automatic plate washer (optional), A luminescence microplate reader

Optional

Decomplexing Buffer - SKU: UE-1003-2000. This buffer is available for purchase on the LifeSensors website **here**.

For some targets, the decomplexing agent enhances the signal to background. We recommend the use of the decomplexing agent prior to analysis with PA950, PA480 & PA630. The use of the decomplexing agent disrupts any native protein complexes that might be part of ubiquitin complexes. This urea based decomplexing agent results in reduced background signal, resulting in a better signal-to-background ratio.

Detailed protocol

1. Remove plate from freezer and reagents from refrigerator and allow them to reach room temperature (RT). All incubations are performed at RT (22°-27°C). To avoid cross contamination, do not re-use plate sealers.
2. Prepare cell lysate and use 15-25 µg/well diluted with PBS (V_t = 50-100 µl/well).
3. Incubate plate for 1.5 - 2 hours at RT with shaking.
4. Wash plate with PBST (4 x 200 µL/well). After the last wash, remove the residual buffer by lightly tapping the plate (upside down) on paper towels or other blotting paper. DO NOT ALLOW WELLS TO DRY COMPLETELY.
5. Dilute primary antibody in 1x Blocking concentrate (dilution depends on primary antibody efficiency; a good starting dilution is 0.5-1 µg/mL) and add 100 µL/well and incubate plate for 1 hours at RT with shaking.
6. Repeat washing as described above (see step 4).
7. Dilute secondary HRP-conjugated antibody in 1x blocking concentrate (dilution as recommended by manufacturer) and add 50-100 µL/well and incubate plate for 30-45 minutes at room temperature with shaking.
8. Repeat washing as described above (see step 4).
9. Just before use, mix 800 µL of DR1 and 800 µL of DR2 into 10 mL of ultrapure water (deionized or distilled). Add 50-100 µL of this solution to each well and read with a plate reader optimized for chemiluminescence for 5-10 reads with 1-minute intervals.

Notes

1. For Targeted protein degradation applications - to monitor K48 polyubiquitination of target protein followed by its degradation, please select times before and after Dmax of PROTAC.
2. Optimize antibody dilution and specificity of target protein by western blotting. Selection of target antibody should be suitable for sandwich ELISA-based assays.
3. Optimize lysate concentration depending on target abundance.
4. See suggested lysis buffer below to have the best polyubiquitination profile of target protein.
5. Include appropriate controls for best results and interpretation.

Cell lysis protocol

1. Aspirate medium completely and rinse cells with ice cold 1X PBS. Scrape cells appropriately with 1X PBS, centrifuge to pellet cells and remove PBS.
2. Freeze cell pellet at -80°C for long term storage or proceed with lysis on ice.
3. Add RIPA lysis buffer (5-10 times the volume of the pellet, (i.e. 100 µL of pellet add 500-1000 µL lysis buffer). Vortex intermittently for 10-15 minutes while keeping the sample on ice to allow efficient lysis.

RIPA lysis buffer needs to be supplemented with UPS inhibitor cocktail to preserve ubiquitination signature and generate robust signal-to-background.

4. Centrifuge at 13,000 xg for 15-20 minutes at 4°C.
5. Collect the supernatant (lysate) and determine protein concentration using standard methods.

RIPA Lysis Buffer

50 mM Tris-HCl, pH 7.5

150 mM NaCl

1% NP40

1% Sodium deoxycholate

2 mM EDTA

1 mM PMSF

10% Glycerol

Aprotinin (10 mg/ml stock) 20 µL/10 mL

1X UPS inhibitor cocktail

Representative Data from PA480 - K48 Ubiquitin Linkage ELISA Kit:

(A) (B)

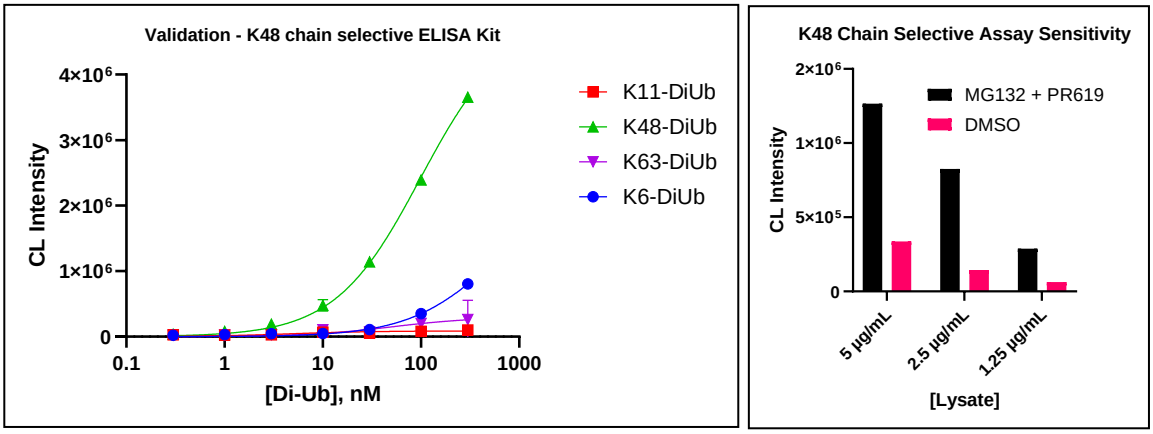


Figure 1: Validation of LifeSensors K48 Ubiquitin linkage ELISA Kit (A) Dose response of K6, K11, K48, and K63 biotinylated Di-Ubiquitin chains captured on ELISA plates coated with K48 selective capture reagent detected using streptavidin HRP. (B) Sensitivity of LifeSensors K48 ubiquitin linkage ELISA Kit in cellular lysates. Cellular lysates derived from HeLa cells treated with 3µM MG-132 and PR-619 along with DMSO as vehicle control were evaluated. Detection of cellular K48 ubiquitination was performed using K48 TUBE HF Biotin (UM307) and streptavidin HRP. Robust signal-to-background between DMSO control and proteasome inhibitor treated samples even at 1.25 µg/mL of lysate concentration represent sensitivity of the ELISA Kit. Error bars represent standard deviation, n=3. **See application note for detailed experimental plan and assay conditions**.

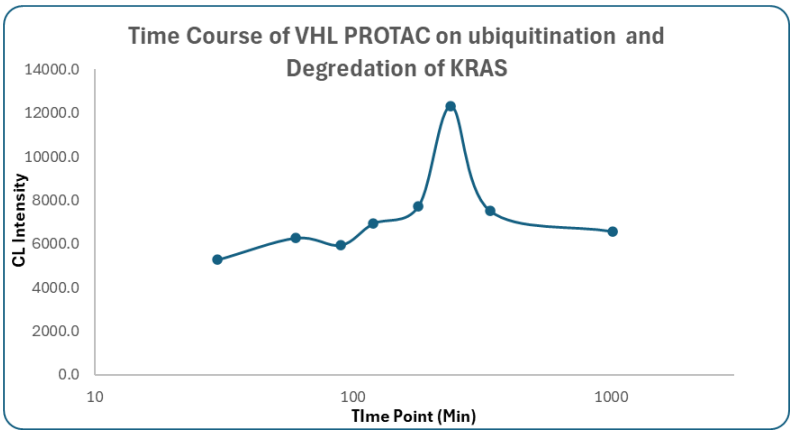


Figure 2: Validation of KRAS PROTACs using LifeSensors K48 Ubiquitin linkage ELISA Kit. H358 Cells were treated with PROTAC (1 µM) in a time course study with times ranging from 0 to 1020 minutes. Cell lysates derived using conditions previously described followed by loading 15 µg/well were processed as described in the manual Results demonstrate enhancement in chemiluminescent intensity by 2 hours represent K48 ubiquitination on KRAS reached maximum followed by protein turnover. Error bars represent standard deviation, n=3. **See application note for detailed experimental plan and assay conditions**.