Poly-Ubiquitinated Protein ELISA Kit

C1375212

Storage -20℃, 2-8℃, Room Temperature. Store in the dark. For detailed storage information, please refer to the kit contents.

C1375212D, C1375212J: are sterile solutions, store at 2-8℃ for up to 1 year. Once the container is opened, use as soon as possible. Stored at -20℃ for long-term.

C1375212B, C1375212E, C1375212F, C1375212G, store at -20℃, avoid freeze/thaw cycle.

Shipping Shipped under ultra-low temperature and low temperature conditions. Please store under the specified storage conditions immediately upon receipt.

Introduction

Ubiquitin (Ub)-mediated post-translational modification is a core regulatory pathway that participates in virtually all cellular physiological activities. Beyond its canonical function in proteasomal degradation, ubiquitin is now known to be involved in signal transduction, DNA damage response, intracellular protein trafficking, cell-cycle regulation, inflammatory responses, immune responses, and apoptosis regulation. Ubiquitin modification is achieved through an isopeptide bond: the C-terminus of ubiquitin is covalently conjugated to the ε-amino group of a lysine residue on the target protein, a process termed ubiquitination. Ubiquitin itself contains seven lysine residues (K6, K11, K27, K29, K33, K48, and K63), each of which can link to another ubiquitin molecule, forming polyubiquitin chains. Different lysine-linked chain types confer diverse regulatory functions upon the ubiquitin system. The two best-characterised polyubiquitin chain types are K48- and K63-linked chains: K48-linked chains primarily target proteins for proteasomal degradation, whereas K63-linked chains regulate protein function, subcellular localisation, and protein-protein interactions. Substantial evidence demonstrates that K11-linked chains are involved in mitotic regulation, and research on this chain type is expected to expand to a scale comparable to that of K48 and K63 in the future. As more associations between other chain types and diseases or specific signalling pathways are reported, interest in the less common ubiquitin chains will rapidly increase. Ubiquitination is a dynamic and reversible process. E1 activating enzymes, E2 conjugating enzymes, and E3 ligases cooperate to catalyse mono- or polyubiquitination, whereas deubiquitinases (DUBs) can remove mono-/polyubiquitin moieties from proteins and hydrolyse ubiquitin chains.

This polyubiquitinated protein ELISA kit is designed for enrichment and detection of polyubiquitinated protein complexes. In the absence of an equivalent absolute quantitative standard for polyubiquitinated proteins, the kit provides a cell-lysis matrix reference solution, the total protein of which has been determined by the BCA method. This reference is intended solely for correction of experimental system deviations and uniform sample loading, and is suitable for relative comparison of ubiquitination levels among multiple cell sample groups within the same plate and same batch. The system exhibits extremely high detection sensitivity:

the minimum positive detection threshold is 0.5 μg/mL based on total protein input, and it maintains good concentration resolution near this threshold (effectively distinguishing samples with total protein concentration differences as low as 0.25 μg/mL). This feature makes the kit particularly suitable for dynamic monitoring of polyubiquitination levels in micro-samples or low-abundance primary cells. Data are recommended to be compared statistically between groups as "normalised OD value/μg total protein".

Detection Principle

Sandwich ELISA system: 96-well plate is coated with a capture reagent for ubiquitinated proteins, which binds ubiquitinated protein complexes in the sample. After washing to remove unbound contaminating proteins, a biotinylated detection probe is added. Following another wash to remove free detection probe, horseradish peroxidase (HRP)-labelled streptavidin (SA) is added to catalyse chemiluminescence. Finally, the chemiluminescent substrate is added, and the enzymatic signal intensity is positively correlated with the concentration of polyubiquitinated proteins in the sample. The standard provided with this kit is a specific cell lysate and is intended for qualitative or semi-quantitative screening only. For accurate relative quantification of samples from different cell sources, users are advised to perform matrix-effect validation.

Kit Contents

C1375212	Components	Appearance	96 T	Storage	Quantity Per Test
C1375212A	96 well plate	Solid	1 EA	RT	As needed
C1375212B	Capture Reagent	Colourless liquid	10 μg	-20℃	As needed
C1375212C	Coating Buffer	Colourless liquid	20 mL	2-8℃	As needed
C1375212D	Blocking Buffer	Light-yellow or yellow liquid	40 mL	2-8℃	As needed
C1375212E	Biotinylated Detection Probe	Colourless liquid	20 μL	-20℃. Store in the dark.	1:1000 dilution, 100 μL/well
C1375212F	SA-HRP	Light brown to brown liquid	10 μL	-20℃. Store in the dark.	1:10000 dilution 100 μL/well
C1375212G	Cell Lysis Matrix	Colourless liquid	200 μg	-20℃. Store in the dark.	As need
C1375212H	10×Wash Buffer	Colourless liquid	100 mL	2-8℃	1:10 dilution 300 μL/well*9 times
C1375212I	Cell Extraction Buffer	Colourless liquid	20 mL	2-8℃	As needed
C1375212J	Dilution Buffer	Light-yellow or yellow liquid	50 mL	2-8℃	As needed

C1375212	Components	Appearance	96 T	Storage	Quantity Per Test
C1375212K	Substrate Reagent A	Colourless liquid	8 mL	2-8℃	As needed
C1375212L	Substrate Reagent B	Colourless liquid	10 mL	2-8℃. Store in the dark.	As needed
C1375212M	Substrate Reagent C	Colourless liquid	2 mL	2-8℃. Store in the dark.	As needed
C1375212N	Stop Solution	Colourless liquid	10 mL	2-8℃. Store in the dark.	50 μL/well

Components required but not supplied

1. Protease inhibitor cocktail and deubiquitinase inhibitors, e.g., PR-619 (DUB inhibitor, Aladdin P126704), 1,10-phenanthroline (Aladdin P111141), N-ethylmaleimide (NEM, Aladdin E100553).
2. Microplate reader capable of dual-wavelength measurement at 450 nm/620 nm, or single wavelength at 450 nm.
3. Precision pipettes with disposable tips.
4. Plate washer (optional).
5. Microplate shaker.
6. Disposable gloves and reagent reservoirs.

Instruction for use

1 Cell Extraction

Note: This protocol has been successfully applied to various cell lines. Users should optimise the extraction procedure for their own applications.

1.1 Collect cells by centrifugation (non-adherent cells) or scraping (adherent cells) from culture flasks, using PBS.

1.2 Wash cells twice with ice-cold PBS.

1.3 Remove and discard the supernatant, and collect the cell pellet.

1.4 On ice, lyse the cell pellet in cell extraction buffer (0.5 mL) for 30 minutes, vortexing every 10 minutes. As a rough guide, adjust the cell concentration to approximately 2×10^7 cells/mL. With this lysis buffer, the protein concentration of the lysate should be 2-4 mg/mL. Note: It is strongly recommended to supplement the cell extraction buffer with protease inhibitor cocktail and deubiquitinase inhibitors (e.g., PR-619, 1,10-phenanthroline, NEM) and to perform all steps on ice, to prevent total protein degradation and removal of ubiquitin modifications by deubiquitinases, thereby preserving the integrity of polyubiquitinated protein complexes.

1.5 Transfer the lysate to a microcentrifuge tube and centrifuge at 15,000 rpm for 10 minutes

at 4℃.

1.6 Dispense the clarified extract into clean microcentrifuge tubes. The lysates can be used directly for the assay or stored at -80℃. Avoid repeated freeze-thaw cycles.

2 ELISA

The volume of each reagent can be calculated proportionally based on the number of samples, replicate layout and dilution series. All reagents should be equilibrated to room temperature prior to the assay.

2.1 Preparation of Wash Buffer

Add 100 mL of 10× Wash Buffer to 900 mL of deionized water and mix thoroughly to prepare working wash solution. Store at 4 ℃ for short-term use, or at -20 ℃ for long-term storage.

2.2 Coating

Dilute capture reagent with coating buffer to the recommended concentration of 0.5 μg/mL and mix well for later use. Take an appropriate number of microplates and place them on plate holders. Add 100 μL of the prepared coating solution to each well, ensuring an even liquid layer at the well bottom without air bubbles. Incubate overnight at 4 ℃.

2.3 Blocking

Discard the coating solution from wells. Add 300 μL wash buffer to each well for one wash cycle either by microplate washer or manual washing, then tap the plate dry. Add 200 μL blocking buffer to each well and incubate for 2 h at 37 ℃, or overnight at 4 ℃.

2.4 Sample Preparation

Dilute all samples with dilution buffer to uniform total protein concentrations (e.g., 100, 50, 25, 10, 5, 2, 1, 0.5, 0.25, 0.125 μg/mL) to maintain consistent loading volume. Negative controls (e.g., dilution buffer, cell extraction buffer) and positive controls (cell lysate matrix) are required. Dilution factors should be optimized individually for each experimental system.

2.5 Sample Loading

Add 100 μL of diluted samples, negative controls and positive controls to corresponding wells. Running 2-3 technical replicates is recommended to reduce experimental deviation. Both negative and positive controls must be included in every assay run. Incubate at 37 ℃ for 1 h.

2.6 Detection Probe Incubation

Wash each well three times with 300 μL wash buffer per well using a microplate washer or manually, then tap dry. Prepare sufficient biotinylated detection probe as required, dilute the probe 1:1000 with dilution buffer, and dispense 100 μL diluted probe into each well. Cover the microplate with aluminum foil or equivalent lightproof material to avoid light exposure, then incubate at 37 ℃ for 1 h.

2.7 Streptavidin Conjugate Incubation

Wash each well three times with 300 μL wash buffer per well using a microplate washer or manually, then tap dry. Prepare adequate HRP-conjugated streptavidin (SA-HRP) as needed, dilute SA-HRP 1:10000 with dilution buffer, and add 100 μL diluted SA-HRP to each well. Cover the microplate with aluminum foil or equivalent lightproof material to avoid light exposure, then incubate at 37 ℃ for 1 h.

2.8 Color Development

Wash each well three times with 300 µL wash buffer per well using a microplate washer or manually, then tap dry. Prepare chromogenic substrate solution right before use by mixing Substrate A, Substrate B and Substrate C at a volume ratio of 4:5:1. Add 100 µL substrate solution to each well. Shield the plate from light with aluminum foil and incubate at 37 ℃ for 5-10 min.

2.9 Reaction Stopping

Once adequate color development is observed, add 50 µL stop solution to each well to halt the reaction.

2.10 Plate Reading

Measure the absorbance of each well on a microplate reader using dual wavelengths at 450 nm and 630 nm. Plate reading must be carried out promptly after stop solution addition.

Precautions

1. Although we recommend the protocol described above, optimal experimental conditions may vary depending on the parameters under study and must be determined by the user.
2. Before starting the ELISA, bring all kit components to room temperature.
3. Blocking Buffer (Cat. No. C1375212D) and Dilution Buffer (Cat. No. C1375212J) are sterile solutions containing nutrients. Please pay attention to sterile operation. After opening, please use it as soon as possible to avoid affecting the subsequent experimental results.
4. The plate is supplied as break-apart strips, allowing partial use. Since experimental conditions may vary, include one aliquot of the cell-lysis matrix reference as a calibrator in each assay run.
5. Use disposable tips and reagent reservoirs for all liquid transfers to avoid cross-contamination between reagents or samples.
6. Wash thoroughly with wash buffer and tap dry completely.
7. Use the highest-grade deionised water.
8. Avoid contact with substrate solutions containing hydrogen peroxide and with the acidic stop solution. The stop solution contains sulfuric acid and is a strong acid. Wear disposable gloves and eye protection when handling. In case of contact with stop solution or substrate solution, rinse the affected skin immediately with plenty of water and seek medical attention if necessary.