Polyubiquitin-B (UBB) ELISA Kit

Catalogue No.:abx153546

Polyubiquitin-B (UBB) ELISA Kit is an ELISA Kit for the in vitro quantitative measurement of Human, Mouse, Rat, Rabbit, Cow, Dog, Pig, Horse Polyubiquitin-B (UBB) concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids. This assay has high sensitivity and excellent specificity for detection of Polyubiquitin-B (UBB)
No significant cross-reactivity or interference between Polyubiquitin-B (UBB) and analogues was observed.

For Reference Only

Target:	Polyubiquitin-B (UBB)
Research Area:	Reproductive Science, Neuroscience, Developmental Science
Reactivity:	Human, Mouse, Rat, Rabbit, Cow, Dog, Pig, Horse
Tested Applications:	ELISA
Recommended dilutions:	Optimal dilutions/concentrations should be determined by the end user.
Storage:	Shipped at 4°C. Upon receipt, store the kit according to the storage instruction in the kit's manual.
Validity:	The validity for this kit is at least 6 months. Up to 12 months validity can be provided on request.
Stability:	The stability of the kit is determined by the rate of activity loss. The loss rate is less than 5% within the expiration date under appropriate storage conditions. To minimize performance fluctuations, operation procedures and lab conditions should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same user throughout.
Gene Symbol:	UBB
Test Range:	4.94 ng/ml - 400 ng/ml
Sensitivity:	< 1.70 ng/ml
Standard Form:	Lyophilized
Detection Method:	Colorimetric
Assay Type:	Competitive
Assay Data:	Quantitative
Sample Type:	Serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

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Target Type: Antigen

Assay Principle: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the biotin-labelled Ub and the unlabelled- Ub on the pre-coated antibody. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Ub will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the color yellow is inversely proportional to the Ub amount bound on the plate. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of Ub can be calculated.

Kit Components: The kit components listed are for reference only. The product manual may differ slightly. The product should be used as stated on the product manual included and delivered together with the product.

- Pre-coated 96-Well Microplate
- Standard
- Standard Diluent Buffer
- Wash Buffer
- Detection Reagent A
- Detection Reagent B
- Diluent A
- Diluent B
- TMB Substrate
- Stop Solution
- Plate Sealer

Material Required But Not Provided:

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

Reagent Preparation: This procedure is provided for reference only. The product manual may differ slightly. The product should be used as stated on the product manual included and delivered together with the product.

- 1) Standard: Prepare the standard with the recommended volume of Standard Diluent Buffer, to make the standard solution. Then use the Standard Diluent buffer to carry out serial dilutions of the standard solution, as instructed in the Protocol.
- 2) Wash Buffer: Dilute the concentrated Wash Buffer with distilled water, as instructed in the Protocol.
- 3) Detection Reagent Preparation: Calculate the total volume of working solution required. Dilute Detection Reagent A and Detection Reagent B with Diluent A and Diluent B, respectively, at 1:100.

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Assay Procedure:

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- 1) Set standard, test samples and control wells.
- 2) Aliquot 50 µl of diluted standard into the standard wells.
- 3) Aliquot 50 µl of Standard Diluent buffer into the control (zero) well.
- 4) Aliquot 50 µl of diluted samples into the sample wells.
- 5) Immediately aliquot 50 µl of Detection Reagent A to each well. Incubate for 1 hr at 37 °C.
- 6) Wash 3 times.
- 7) Aliquot 100 µl of Detection Reagent B to each well. Incubate for 30 mins at 37 °C.
- 8) Wash 5 times.
- 9) Aliquot 90 µl of TMB Substrate to each well. Incubate for 10-20 mins at 37 °C.
- 10) Aliquot 50 µl of Stop Solution.
- 11) Measure the OD at 450 nm.

Protocol:

This procedure is provided for reference only. The product manual may differ slightly. The product should be used as stated on the product manual included and delivered together with the product.

- Equilibrate the kit components and samples to room temperature (18 - 25 °C) before use. It is recommended to plot a standard curve for each test.
- 1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample at least in duplicate.
- 2. Add 50 µL of each standard, control and sample into the appropriate wells.
- 3. Remove the cover and discard the liquid.
- 4. Immediately aliquot 50 µl of Detection Reagent A working solution. Seal the plate with a cover and incubate for 1 h at 37°C.
- 5. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer.
- 6. Add 100 µL of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
- 7. Discard the solution and wash the plate 5 times with wash buffer as explained in previous step.
- 8. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
- 9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Results Calculation:

This assay is competitive, therefore there is an inverse correlation between Ub concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and average absorbance measured (x-axis). Apply a best fit trendline through the standard points. The Ub concentration of the samples can be interpolated from the standard curve.

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of Ubiquitin (Ub) were were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of Ubiquitin (Ub) were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

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Note:

THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

The range and sensitivity is subject to change. Please contact us for the latest product information.

For accurate results, sample concentrations must be diluted to mid-range of the kit. If you require a specific range, please contact us in advance or write your request in your order comments.

Please note that our kits are optimised for detection of native samples, rather than recombinant proteins. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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