Mouse Bcl2 Associated X Protein (BAX) ELISA Kit

Catalogue No.:abx153702

Mouse Bcl2 Associated X Protein (BAX) ELISA Kit is an ELISA Kit for the in vitro quantitative measurement of Mouse BAX concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids.. This assay has high sensitivity and excellent specificity for detection of Bcl2 Associated X Protein (BAX)
No significant cross-reactivity or interference between Bcl2 Associated X Protein (BAX) and analogues was observed.

Target:	Bcl2 Associated X Protein (BAX)
Reactivity:	Mouse
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.
Test Range:	62.5 pg/ml - 4000 pg/ml
Sensitivity:	< 25.3 pg/ml
Standard Form:	Lyophilized
Detection Method:	Colorimetric
Assay Type:	Sandwich
Assay Data:	Quantitative
Sample Type:	Serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids.
Target Type:	Antigen

For Reference Only

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Assay Principle:

This kit is based on sandwich 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. 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 BAX will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the BAX amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of BAX 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 100 µl of diluted standard into the standard wells.
- 3) Aliquot 100 µl of Standard Diluent buffer into control (zero) well.
- 4) Aliquot 100 µl of diluted samples into the sample wells. Incubate for 1 hr at 37 °C.
- 5) Aliquot 100 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 100 µL of each standard, control and sample into the appropriate wells. Seal the plate with a cover and incubate for 1 h at 37°C.
- 3. Remove the cover and discard the liquid.
- 4. Add 100 µl of the detection Reagent A working solution to each well. 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:

For calculation, average the O.D.450 duplicate readings for each reference standard and each sample and substract the average control (zero) O.D.450 reading. The standard curve can be plotted as the relative O.D.450 of each reference standard solution [Y] vs. the respective concentration of each standard solution (X). The BAX 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 BAX were were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of BAX 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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