Mouse Thromboxane B2 (TXB2) ELISA Kit

Cat. No.: EJ1515543 | Pack size: 48T, 96T | Storage: 2-8°C

Overview

Thromboxane B2 (TXB2) is an inactive metabolite/product of thromboxane A_2. It is almost completely cleared in urine. It does not participate in platelet activation and aggregation itself, whereas its precursor thromboxane A_2 does. Thromboxane A_2 synthesis is the target of aspirin, which inhibits COX-1 enzyme (the source of thromboxane A_2 in platelets). 2-(3,4-dihydroxyphenyl)ethanol (DHPE) is a phenolic component in extra virgin olive oil. Olive oil fractions containing DHPE can inhibit platelet aggregation and thromboxane B_2 formation in vitro.

Assay Principle

This kit adopts competitive enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotin-labeled antibody and HRP conjugate are sequentially added into microplates pre-coated with mouse thromboxane B2 (TXB2) antigen, followed by incubation and washing steps. Color development is performed with TMB substrate. TMB turns blue under the catalysis of horseradish peroxidase (HRP), and finally converts to yellow after acid termination. The color intensity is negatively correlated with the concentration of mouse thromboxane B2 (TXB2) in samples. The absorbance (OD value) is measured at 450 nm using a microplate reader for calculation of sample concentrations.

Precautions

1. Strictly follow the specified incubation time and temperature to guarantee accurate results. All reagents must be equilibrated to room temperature (20-25°C) before use. Refrigerate reagents immediately after use.
2. Improper plate washing may lead to inaccurate readings. Ensure complete removal of residual liquid in wells prior to substrate addition. Avoid prolonged drying of microwells throughout the procedure.
3. Wipe residual liquid and fingerprints from the bottom of the microplate, as contaminants will interfere with OD values.
4. The TMB substrate solution shall remain colorless; discard substrate solution that has turned blue.
5. Prevent cross-contamination between reagents and samples to avoid erroneous test results.
6. Avoid direct intense light exposure during reagent storage and incubation.
7. All reaction reagents must not come into contact with bleach solvents or their volatile fumes; bleach components will destroy the biological activity of kit reagents.
8. Do not use expired products; components from different lot numbers or batch codes cannot be mixed.
9. Recombinant proteins from external sources may fail to be recognized due to mismatched antibody affinity with kit capture antibodies.

10. Handle all potentially infectious samples properly, and dispose of specimens and testing devices in accordance with standardized biosafety protocols.

Kit Components

EJ1515543	Components	Appearance	48T	96T	Storage
EJ1515543A	Pre-coated Assay Plate	—	48 wells	96 wells	2-8°C
EJ1515543B	Standard	Solid	1 vial	2 vials	2-8°C
EJ1515543C	Universal Diluent	Liquid	1×20 mL	2×20 mL	2-8°C
EJ1515543D	Biotin-antibody (100×)	Liquid	30 µL	60 µL	2-8°C
EJ1515543E	Streptavidin-HRP (100×)	Liquid	60 µL	120 µL	2-8°C
EJ1515543F	Wash Buffer (20×)	Liquid	1×10 mL	2×10 mL	2-8°C
EJ1515543G	TMB Substrate	Liquid	5 mL	10 mL	2-8°C
EJ1515543H	Stop Solution	Liquid	3 mL	6 mL	2-8°C
EJ1515543I	Plate Sealer	—	4 pieces	4 pieces	2-8°C

Note: *EJ1515543B, EJ1515543D, EJ1515543E and EJ1515543F shall be diluted in accordance with the instructions.*

Sample Handling & Requirements

1. The detectable concentration range of the kit does not equal the analyte concentration range in raw samples. It is recommended to estimate sample analyte concentrations via relevant literature and conduct pre-tests to confirm actual concentrations before formal experiments. Dilute or concentrate samples appropriately if analyte levels fall outside the linear range.
2. If your sample matrix is not listed in this manual, perform a pre-test to verify assay compatibility.
3. Serum: Collect whole blood in serum separation tubes, incubate at room temperature for 2 hours or store overnight at 2-8°C, then centrifuge at 1000×g for 20 minutes. Harvest supernatant for immediate testing, or aliquot and store at -20°C / -80°C; avoid repeated freeze-thaw cycles.
4. Plasma: Collect blood with EDTA or heparin as anticoagulant. Centrifuge specimens within 30 minutes post-collection at 1000×g, 2-8°C for 15 minutes. Collect supernatant for immediate testing, or store at -20°C / -80°C; avoid repeated freeze-thaw cycles.

5. Tissue Homogenate: Rinse tissue with pre-cooled PBS (0.01 M, pH=7.4) to remove residual blood (lysed erythrocytes in homogenate interfere with detection results). Weigh and mince tissue, then mix tissue with PBS at a standard weight-to-volume ratio of 1:9 (e.g., 1 g tissue corresponds to 9 mL PBS; adjust volume as required and record details. Addition of protease inhibitor cocktail to PBS is recommended). Transfer the mixture to a glass homogenizer and grind thoroughly on ice, or use a mechanical homogenizer. For complete cell lysis, sonicate the homogenate or perform repeated freeze-thaw cycles. Finally, centrifuge the homogenate at 5000×g for 5-10 minutes and collect supernatant for testing.
6. Cell Culture Supernatant: Centrifuge culture medium at 1000×g for 20 minutes, collect supernatant for immediate testing, or store at -20°C / -80°C; avoid repeated freeze-thaw cycles.
7. Cell Lysate: For adherent cells, wash gently with pre-cooled PBS, digest with trypsin, then harvest cells by centrifugation at 1000×g for 5 minutes. Suspension cells can be pelleted directly by centrifugation. Wash harvested cells 3 times with pre-cooled PBS, resuspend 1×10^6 cells in 150-200 µL PBS (protease inhibitors are recommended; reduce PBS volume if analyte abundance is extremely low). Lyse cells via repeated freeze-thaw or sonication. Centrifuge the lysate at 1500×g, 2-8°C for 10 minutes and retain the supernatant for detection.
8. Other Biological Matrices: Centrifuge samples at 1000×g for 20 minutes and collect supernatant for testing.
9. Sample Appearance: Samples must be clear and transparent; all suspended particulates must be removed by centrifugation.
10. Sample Storage: Samples intended for testing within 1 week can be stored at 4°C. For delayed testing, aliquot samples into single-use volumes and freeze at -20°C (stable for 1 month) or -80°C (stable for 6 months). Avoid repeated freeze-thaw cycles. Hemolyzed samples are unsuitable for this assay, as hemolysis will distort final detection results.

Sample Dilution Protocols

Estimate analyte concentration in advance. Follow the dilution schemes below if sample dilution is required:

100-fold dilution (single step): Mix 5 µL sample with 495 µL Assay Diluent.

1000-fold dilution (two-step): Step 1: Dilute 5 µL sample into 95 µL diluent (20×). Step 2: Transfer 5 µL of the 20× diluted sample into 245 µL diluent (50×), achieving total 1000-fold dilution.

100000-fold dilution (three-step): Step 1: Mix 5 µL sample with 195 µL diluent (40×). Step 2: Transfer 5 µL 40× sample into 245 µL diluent (50×). Step 3: Transfer 5 µL 2000× sample into 245 µL diluent (50×), total dilution factor = 100000×.

Guidelines for dilution: Transfer volume per step ≥ 3 µL; single-step dilution factor ≤ 100×. Mix thoroughly after each dilution and avoid bubble formation.

Required Lab Equipment (User-Supplied)

1. Microplate reader (capable of 450 nm absorbance measurement).
2. High-precision pipettes and matching tips: 0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL.

3. 37°C incubator.
4. Distilled or deionized water.

Pre-Assay Reagent Preparation

1. Remove the kit from refrigeration 10 minutes in advance and equilibrate to room temperature.
2. Preparation of Serial Standard Working Solution: Reconstitute the lyophilized standard with 1 mL assay diluent, leave it to stand for 15 minutes for complete dissolution, then mix gently (stock concentration = 5000 pg/mL). Prepare serial dilutions at concentrations of: 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.25 pg/mL, 78.12 pg/mL, 0 pg/mL.

Serial dilution procedure: Prepare 7 EP tubes and add 500 µL assay diluent to each tube. Transfer 500 µL of the 5000 pg/mL standard working solution into the first tube and mix well to obtain a 2500 pg/mL standard working solution, then perform serial transfer and mixing in sequence for subsequent tubes. The last tube serves directly as the blank well; no liquid needs to be transferred from the penultimate tube. The serial dilution scheme is shown in Figure 1.

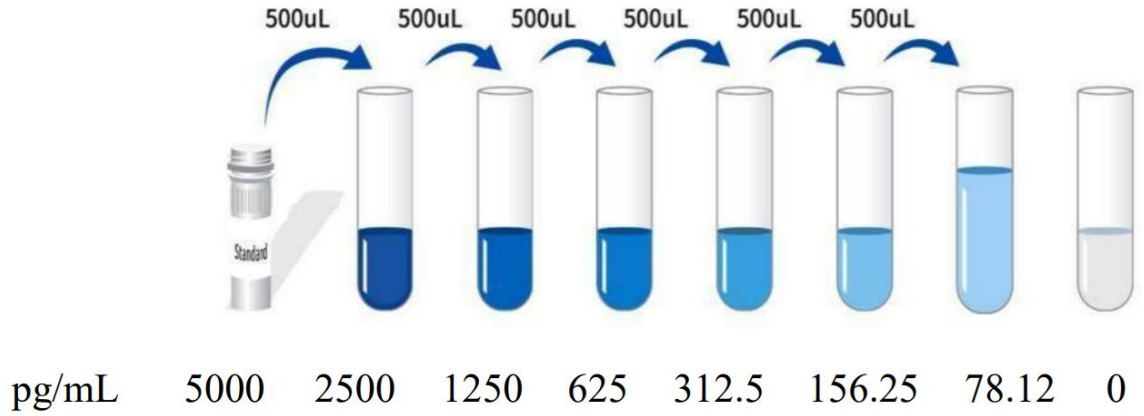

Figure 1. Serial Standard Dilution Scheme

3. Preparation of Biotin-antibody Working Solution: Centrifuge the 100× concentrated Biotin-antibody at 1000×g for 1 minute 15 minutes before use. Dilute the 100× concentrated Biotin-antibody to 1× working concentration with assay diluent (Example: 10 µL concentrate + 990 µL assay diluent). Prepare fresh right before use.
4. Preparation of Streptavidin-HRP Working Solution: Centrifuge the 100× concentrated Streptavidin-HRP at 1000×g for 1 minute 15 minutes before use. Dilute the 100× concentrated Streptavidin-HRP to 1× working concentration with assay diluent (Example: 10 µL concentrate + 990 µL assay diluent). Prepare fresh right before use.
5. Preparation of 1× Wash Buffer: Add 10 mL of 20× concentrated wash buffer into 190 mL distilled water. (Crystallization may occur in concentrated wash buffer stored in the refrigerator, which is a normal phenomenon. Place the buffer at room temperature and wait for all crystals to dissolve completely before preparation.)

Assay Procedure

1. Remove required strips from the aluminum foil pouch equilibrated at room temperature for 10 min. Seal leftover strips in a zip bag and store at 4°C.
2. Sample Loading: Add 50 µL of samples or standards of different concentrations into corresponding wells. Add 50 µL Universal Diluent to blank wells, then add 50 µL Biotin-antibody working solution to each well. Cover with Plate Sealer and incubate at 37°C for 60 min. (Recommendation: Dilute test samples at least 1-fold with Universal Diluent before loading onto the Pre-coated Assay Plate to reduce matrix effect on results. Multiply the measured concentration by the dilution factor during final calculation. It is recommended to run all test samples and standards in duplicate.)
3. Wash: Discard liquid. Add 300 µL of 1× Wash Buffer to each well, let stand for 1 min, flick out buffer and blot dry on absorbent paper. Repeat the wash procedure 3 times (automated plate washer is acceptable).
4. Add Conjugate Working Solution: Add 100 µL Streptavidin-HRP working solution to each well. Cover with Plate Sealer and incubate at 37°C for 30 min.
5. Wash: Discard liquid and repeat the washing procedure described in Step 3 for 5 times.
6. Add Substrate: Add 90 µL TMB Substrate to each well. Cover with Plate Sealer and incubate at 37°C in the dark for 15 min.
7. Add Stop Solution: Take out the assay plate, add 50 µL Stop Solution directly into each well. Immediately measure OD values of each well at 450 nm.

Data Analysis

1. Calculate the average OD values of replicate wells for standards and samples, then subtract the OD value of blank wells to obtain corrected OD values. Plot a 4-parameter logistic standard curve on log-log graph paper with concentration as the X-axis and corrected OD value as the Y-axis.
2. If the OD value of a sample exceeds the upper limit of the standard curve, dilute the sample appropriately and re-test. Multiply the measured concentration by the corresponding dilution factor for final calculation.

Reference Standard Curve Raw Data (For Illustration Only)

All data and curves below are for reference only; users must generate a standard curve based on their own experimental runs.

Concentration (pg/mL)	5000	2500	1250	625	312.5	156.25	78.12	0
OD Value	0.188	0.270	0.389	0.642	0.929	1.380	1.766	1.842

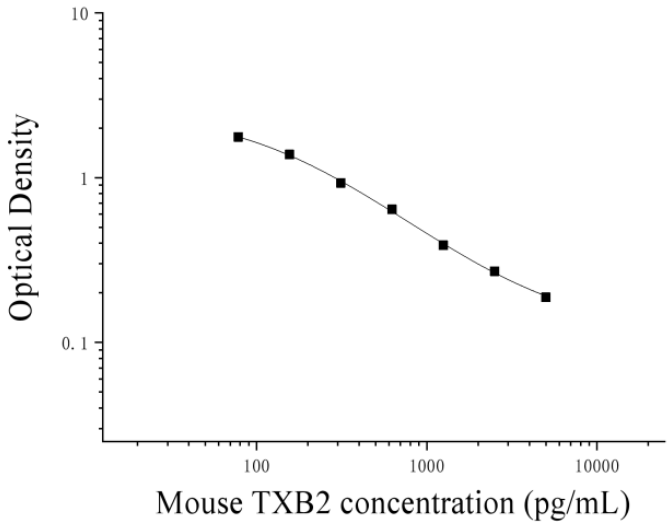

Figure 2. Reference Standard Curve for Mouse TXB2 ELISA

Note: *Figure 2 is for reference only; sample concentration shall be calculated based on the standard curve generated from experimental data of each individual test run.*

Kit Performance Specifications

1. Precision: Intra-assay coefficient of variation (CV) < 10%, inter-assay CV < 10%.
2. Recovery: Three distinct concentration levels of mouse TXB2 were spiked into selected healthy mouse serum and plasma samples, and the recovery rates were calculated.

Sample Type	Range (%)	Average Recovery Rate (%)
Serum (n=8)	81-102	97
Plasma (n=8)	92-105	101

3. Linearity by Dilution: High-concentration mouse TXB2 was spiked into four selected healthy mouse serum and plasma samples respectively. Serial dilutions were prepared within the dynamic range of the standard curve to evaluate assay linearity.

Dilution Ratio	Recovery Index	Serum	Plasma
1:2	Range (%)	83-96	88-96
1:2	Average Recovery Rate (%)	92	93
1:4	Range (%)	89-104	87-108
1:4	Average Recovery Rate (%)	93	98

Troubleshooting Guide

If unsatisfactory assay results occur, take photos of color development, save experimental data, retain used microplate strips and unused reagents, then contact our technical support for troubleshooting. Refer to the troubleshooting items below:

1. Poor Standard Curve Linearity

① Incorrect standard dilution: Reconstitute and dilute standard strictly following recommended protocol.
② Inaccurate pipetting: Calibrate pipettes regularly and verify tip tightness/sealing performance.
③ Evaporation of reaction solution: Seal microplate with plate sealer film.
④ Incomplete washing: Perform sufficient wash cycles with adequate volume of wash buffer.
⑤ Foreign residues at well bottom: Wipe microplate bottom prior to absorbance measurement.

2. Faint or Absent Color Development

① Insufficient incubation duration: Adhere to specified incubation time.
② Incorrect incubation temperature: Incubate at recommended temperature.
③ Insufficient reagent addition volume: Verify pipette accuracy and follow operating procedure strictly.
④ Improper reagent dilution: Double-check dilution steps for all reagents.
⑤ Inactivated Streptavidin-HRP: Mix Streptavidin-HRP with substrate and verify activity via color reaction test.

3. Low OD Readings

① Wrong microplate reader parameter: Confirm detection wavelength setting on instrument.
② Missing stop solution addition: Add appropriate volume of stop solution.
③ Overlong delay before plate reading: Measure absorbance immediately after termination.
④ Excessively high analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.
⑤ Too low analyte concentration in sample: Determine optimal dilution factor via preliminary pre-test.

4. High Background Signal

① Contaminated TMB substrate: Replace with fresh substrate solution.
② Excess substrate incubation time: Strictly control color development duration.
③ Wrong dilution for detection antibody or Streptavidin-HRP: Dilute reagents in accordance with official recommended protocol.
④ Incomplete plate washing: Carry out adequate wash cycles with sufficient wash buffer volume.

Specifications &Storage &Shipping

Item	Information
Grade & Purity	BioReagent
Application	Enzyme immunoassay (ELISA)
Storage	Store at 2-8°C.
Shipped In	Wet ice
Stability and Storage	Store at 2-8°C long term (6 months).

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