Taurine Assay Kit (ELISA)

Cat. No. KITG-143 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit uses a competitive inhibition enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of taurine content in serum, plasma, and other related biological fluids. Taurine antibody is coated on the microwells to prepare the solid-phase carrier, and biotin-labeled antigen together with the antigen to be tested (standard or sample) is added simultaneously to the antibody-coated microwells, where the test antigen and the biotin-labeled antigen compete for binding to the specific antibody. After incubation, unbound material is removed by washing, and HRP-labeled avidin is then added. Following incubation and thorough washing, TMB substrate is added for color development. TMB is converted to blue under peroxidase catalysis and to the final yellow under the action of acid. The higher the concentration of the analyte in the sample, the more the binding of the labeled antigen to the antibody is inhibited and the lighter the color developed. Color intensity is positively correlated with the amount of enzyme and negatively correlated with the taurine content of the sample. Absorbance (OD) is measured at 450 nm with a microplate reader, and the sample concentration is calculated.
Species	Pan-species
Conjugation/Label	Biotin-labeled antigen; HRP-labeled avidin
Description	Sample Collection, Processing, and Storage Serum: Allow whole blood collected in a serum separator tube to stand at room temperature for 2 hours or overnight at 4 °C, then centrifuge at 1,000 × g for 20 minutes. Collect the supernatant and store it at -20 °C or -80 °C; avoid repeated freeze-thaw cycles. Plasma: Collect specimens using

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	EDTA or heparin as the anticoagulant and centrifuge at 1,000 × g for 15 minutes at 2-8 °C within 30 minutes of collection. Collect the supernatant for detection, or store it at -20 °C or -80 °C; avoid repeated freeze-thaw cycles. Other biological specimens: Centrifuge at 1,000 × g for 20 minutes. Collect the supernatant for detection, or store it at -20 °C or -80 °C; avoid repeated freeze-thaw cycles. Sample storage: All specimens listed above must be stored sealed — less than 1 week at 4 °C, no more than 1 month at -20 °C, and no more than 2 months at -80 °C. Notes on samples: Hemolyzed specimens will affect the final result and are not suitable for this assay. Specimens should be slowly equilibrated to room temperature before use and should not be thawed by heating. Serum is recommended over plasma as the test sample.
Form	Size 96 Tests Kit Components Pre-coated 96-well plate: 1 plate Plate sealer: 4 sheets Standard: 2 vials Standard diluent: 1 × 20 mL Detection Solution A: 1 vial Detection Diluent A: 1 × 12 mL Detection Solution B: 1 × 120 µ Detection Diluent B: 1 × 12 mL Reagent diluent: 1 × 300 µ Stop solution: 1 × 6 mL TMB substrate: 1 × 9 mL Concentrated wash buffer (30×): 1 × 20 mL Instruction manual: 1 copy
Bio-activity	Kit Performance Detection range: 12.35-1,000 ng/mL. Sensitivity: The minimum detectable concentration is 4.39 ng/mL. This value corresponds to the concentration calculated from the mean of 20 blank samples (standard diluent) minus two standard deviations. Specificity: This kit is designed for the detection of taurine, and no significant cross-reactivity with other similar substances has been observed. Precision: Expressed as the coefficient of variation (CV) of the measured sample values; CV (%) = SD/mean × 100. Intra-assay CV < 10%; inter-assay CV < 12%. Recovery A defined amount of taurine was added to fixed-value serum and plasma samples, and the samples were repeatedly measured to calculate the mean value. Recovery is the ratio of the measured value to the theoretical value. Serum (n = 5): recovery range 78-92%, mean recovery 85% EDTA plasma (n = 5): recovery range 80-99%, mean recovery 93% Heparin plasma (n = 5): recovery range 91-103%, mean recovery 97% Linearity An appropriate amount of taurine was added to fixed-value

	serum and plasma samples, which were then serially diluted 1:2, 1:4, 1:8, and 1:16. The linear range is the ratio of the measured value to the theoretical value for the taurine content of the diluted samples. Serum (n = 5): 1:2, 83-98%; 1:4, 80-94%; 1:8, 96-106%; 1:16, 81-90% EDTA plasma (n = 5): 1:2, 82-102%; 1:4, 85-101%; 1:8, 79-104%; 1:16, 83-93% Heparin plasma (n = 5): 1:2, 90-105%; 1:4, 82-97%; 1:8, 84-96%; 1:16, 90-99% Stability When the kit is stored at the recommended temperature within its validity period, the activity reduction rate is less than 5%.
Applications	Applications Quantitative determination of taurine content in serum, plasma, and othe r related biological fluids. Detection Method Competitive inhibition enzyme-linked imm unosorbent assay (ELISA); colorimetric detection at 450 nm. Target Species Pan-spe cies Compatible Sample Types Serum; plasma; other related biological fluids.
Notes	Operating Precautions 1. Reagent preparation: Before use, bring all reagents and specimens slowly to room temperature (18-25 °C). If the kit will not be used for a period of time, remove only the plate strips and reagents required for the current run and store the remaining strips and reagents under the specified conditions. The standard must not be diluted directly in the plate. Prepare the standard within 15 minutes before use; it is for single use only. Prepare the standard, Detection Solution A working solution, and Detection Solution B working solution with their corresponding diluents and do not confuse them. Mix gently but thoroughly and avoid foaming. Do not reuse diluted standard, Detection Solution A working solution, or Detection Solution B working solution. 2. Pipetting: Use disposable tips to avoid cross-contamination. Add samples to the bottom of the well without touching the well wall, and avoid bubbles. Keep the total sample-loading time (including standards and all samples) within 10 minutes; replicate wells are recommended. 3. Incubation: To prevent sample evaporation, place the covered or sealed plate in a humidity chamber during the assay. Proceed to the next step as soon as possible after washing, and never allow the plate to dry out at any stage. Strictly observe the specified incubation times and temperatures. 4. Washing: Adequate washing is essential. Completely

remove the wash solution at every washing step. After washing, blot the residual liquid in the reaction wells on filter paper; do not place filter paper directly into the wells to absorb liquid. Remove residual liquid and fingerprints from the underside of the plate. 5. Reaction time control: After adding the substrate, observe the color change in the reaction wells periodically (for example, every 10 minutes). If the color is too dark, add stop solution earlier to avoid an overly strong reaction that affects the OD reading. 6. Substrate: Store the substrate protected from light and avoid direct strong-light exposure during storage and incubation. 7. If the laboratory humidity is below 60%, the use of a humidifier is recommended. Specimen Handling The concentration of the analyte in the sample should be estimated before the assay. When the concentration falls outside the range of the standard curve, the optimal sample dilution factor must be determined for the specific experiment; dilute or concentrate the sample appropriately. Sample dilution must be performed with PBS. If the sample type is not listed in the instructions, a preliminary experiment is recommended to verify applicability, and an aliquot of the sample should be retained. Tissue homogenates or cell extracts prepared with chemical lysis buffers may introduce chemical substances that cause deviation in the ELISA result. Cell culture supernatant is subject to many interfering factors — such as cell state, cell number, and sampling time — and may therefore be undetectable. Some natural or recombinant proteins may not be detected if they do not match the detection and capture antibodies used in this product. Fresh samples are recommended, since prolonged storage may lead to protein degradation or denaturation and cause deviation in the results. Troubleshooting Poor standard curve: The standard was prepared incorrectly — perform the correct serial dilution of the standard; aspiration and washing were insufficient — aspirate and wash adequately; pipetting was inaccurate — check and calibrate the pipette. Low precision: Washing was insufficient — wash and soak adequately as instructed; mixing was inadequate or reagent aspiration was insufficient — mix thoroughly and aspirate reagents completely; tips, containers, and plate sealers were reused — use a new tip for each addition and use fresh containers and plate sealers; sample loading was inaccurate — check and

	calibrate the pipette. Low OD value: The reagent volume added per well was inaccurate — calibrate the pipette and add reagents accurately; the incubation time was incorrect — ensure a sufficient incubation time; the incubation temperature was incorrect — equilibrate reagents to room temperature and ensure an accurate incubation temperature; the enzyme conjugate or substrate has lost activity — check by mixing the enzyme conjugate with the substrate, which should develop color rapidly; no stop solution was added — add stop solution according to the assay procedure; reading was performed outside the recommended reading time — read within the time recommended in the instructions. Sample value issues: Incorrect sample storage — store samples correctly and use fresh samples; incorrect sample collection and handling procedures — apply correct collection and handling procedures; the analyte content in the sample is low — use fresh samples and repeat the assay. Additional Notes Tissue homogenates or cell extracts prepared with chemical lysis buffers, certain recombinant proteins, and cell culture supernatants may not be detected or may show deviation. Reagents supplied in this kit are intended for use as a matched set and must not be mixed with reagents from other sources; optimal results are obtained only by strictly following the instructions provided.
Storage	Storage Unopened kit: The unopened kit may be stored as a whole at -20 °C; storage at 4 °C is limited to 1 month. For convenience, the kit may also be stored disassembled: keep the standard, Detection Solution A, Detection Solution B, and the 96-well plate at -20 °C, and store the remaining reagents at 4 °C. After use: Remaining reagents should still be stored at the temperature indicated on the reagent vial label. In addition, place unused plate strips in an aluminum foil bag containing desiccant, tightly reseal the bag, and store at -20 °C. The plate strips in this kit are detachable and may be used across several runs as required; the remaining kit is recommended to be used within 1 month after the first assay. Stability When the kit is stored at the recommended temperature within its shelf life, all components are guaranteed to be stable, and the activity reduction rate is less than 5%.

Concentration	Standard concentrations (Tube 1 to Tube 6): 1,000, 333.33, 111.11, 37.04, 12.35, and 0 ng/mL. The standard diluent alone (0 ng/mL) serves directly as the blank well. Detection range: 12.35-1,000 ng/mL. Minimum detectable concentration: 4.39 ng/mL.
Reconstitution	Standard (lyophilized): Add 0.5 mL of standard diluent to each vial of standard, cap the vial, and let it stand at room temperature for approximately 10 minutes while swirling gently (avoid foaming) to give a concentration of 1,000 ng/mL. Prepare 5 EP tubes for dilution of the standard, add 600 µ of standard diluent to each tube, and serially dilute 3-fold to obtain 1,000, 333.33, 111.11, 37.04, and 12.35 ng/mL. The standard diluent (0 ng/mL) is used directly as the blank well. Prepare a fresh standard solution for each assay to ensure valid results. Detection Solution A: Add 150 µ of reagent diluent to Detection Solution A, cap the vial, and let it stand at room temperature for approximately 10 minutes, inverting or rubbing the vial repeatedly to aid dissolution. Immediately before use, dilute 1:100 with Detection Diluent A (for example, 10 µ Detection Solution A + 990 µ Detection Diluent A) and mix thoroughly. Prepare the total volume required for each run in advance (50 µ/well), and prepare an extra 0.1-0.2 mL in practice. Detection Solution B: Before use, flick the tube gently or centrifuge it briefly so that liquid on the tube wall or cap collects at the bottom. Immediately before use, dilute 1:100 with Detection Diluent B (for example, 10 µ Detection Solution B + 990 µ Detection Diluent B) and mix thoroughly. Prepare the total volume required for each run in advance (100 µ/well), and prepare an extra 0.1-0.2 mL in practice. Concentrated wash buffer (30×): Dilute 20 mL of concentrated wash buffer to a final volume of 600 mL with 580 mL of distilled or deionized water, i.e., a 30-fold dilution. If crystals have formed in the concentrated wash buffer, warm it to room temperature and mix gently until the crystals are completely dissolved before preparation. Substrate solution: Use a sterile pipette tip to transfer the required volume of TMB into a separate clean container for use. Substrate remaining in that container should be discarded and must not be poured back into the TMB bottle.
Reaction conditions	Plate Washing Discard the liquid in the wells, wash each well with 350 µ of wash

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buffer, soak for 1-2 minutes, and remove all remaining liquid by tapping the plate gently on absorbent paper. Repeat the washing 3 times. After the final wash, aspirate or pour off the remaining wash buffer, invert the plate on absorbent paper, and blot away all liquid remaining in the wells. This process may also be performed with a spray bottle, a multichannel pipette, or an automatic plate washer. Assay Procedure 1. Sample loading: Set up standard wells, sample wells, and a blank well. Set up 5 standard wells and add 50 µ of standard of a different concentration to each (see reagent preparation, step 2). Add 50 µ of standard diluent to the blank well (the last tube in step 2 of reagent preparation), and add 50 µ of the sample to each remaining well. Immediately add 50 µ of Detection Solution A working solution to every well, shake gently to mix, and avoid bubbles. Cover the plate with plate sealer and incubate at 37 °C for 1 hour. 2. Discard the liquid in the wells, wash each well with 350 µ of wash buffer, soak for 1-2 minutes, and remove all liquid by tapping the plate on absorbent paper. Repeat the washing 3 times. After the final wash, aspirate or pour off the remaining wash buffer, invert the plate on absorbent paper, and blot away all residual liquid. A spray bottle, a multichannel pipette, or an automatic plate washer may also be used. 3. Add 100 µ of Detection Solution B working solution (prepared immediately before use) to each well, cover with plate sealer, and incubate at 37 °C for 30 minutes. 4. Discard the liquid in the wells, blot dry, and wash the plate 5 times as described in step 2. 5. Add 90 µ of substrate solution to each well, cover with plate sealer, and develop the color at 37 °C protected from light. Control the reaction time within 10-20 minutes and do not exceed 30 minutes. The reaction may be stopped once the last 3 standard wells show an obvious blue gradient while the first 3 wells show no obvious gradient. 6. Add 50 µ of stop solution to each well to terminate the reaction; the blue color turns yellow immediately. Add the stop solution in the same order as the substrate solution whenever possible. If the color is uneven, swirl the plate gently to mix the solution. 7. After confirming that there are no water droplets on the underside of the plate and no bubbles in the wells, immediately measure the optical density (OD value) of each well with a microplate reader at 450 nm. Assay Time First incubation: 1 hour at 37 °C. Second incubation: 30 minutes at 37 °C. Color

	development: 10-20 minutes at 37 °C in the dark (not exceeding 30 minutes). Result Calculation This kit is based on a competitive inhibition ELISA, so the taurine content of the sample is negatively correlated with color development: the higher the taurine content, the lighter the color, and the lower the content, the darker the color. Plot the OD values of the standards and the samples (five-point plot); if replicate wells are set up, use the mean value for calculation. Construct the standard curve with the standard concentration on the y-axis (logarithmic scale) and the OD value on the x-axis, and calculate the regression equation of the standard curve (the best equation should be selected according to the R^2 value calculated from the regression equation; the closer R^2 is to 1, the better). Substitute the OD value of the sample into the equation to calculate the sample concentration, then multiply by the dilution factor to obtain the actual sample concentration. Typical data note: For convenience of calculation, although concentration is the independent variable and the OD value is the dependent variable, the OD value of the standard is still used as the x-axis and the logarithm of the standard concentration as the y-axis when plotting. Because experimental conditions differ (operator, pipetting technique, washing technique, temperature conditions, and so on), the OD values of the standard curve will vary. The standard curve provided is for reference only, and each user should establish a standard curve from their own experiment. Workflow Summary 1. Prepare the standards, reagents, and samples before the assay. 2. Add 50 µ of standard or sample, then immediately add 50 µ of Detection Solution A and incubate at 37 °C for 1 hour. 3. Blot dry and wash the plate 3 times. 4. Add 100 µ of Detection Solution B and incubate at 37 °C for 30 minutes. 5. Wash the plate 5 times. 6. Add 90 µ of TMB substrate and incubate at 37 °C for 10-20 minutes. 7. Add 50 µ of stop solution and read at 450 nm immediately.
Usage	For research use only. Not for use in diagnostic procedures. Required Materials and Equipment Microplate reader equipped with a 450 ± 10 nm filter (preheating of the instrument before use is recommended) Single-channel or multichannel micropipettes and tips EP tubes for sample dilution Distilled or deionized water Absorbent paper

	Container for wash solution 0.01 mol/L (1×) phosphate-buffered saline (PBS), pH 7.0-7.2

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