ATP Content Detection Kit (ELISA)

Cat. No. KITG-050 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The ATP Content Detection Kit (ELISA) is designed for the quantitative determination of Adenosine Triphosphate (ATP) in human serum, plasma, cell culture supernatant, and tissue homogenate samples. The kit employs a double-antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA). Pre-coated microplate wells with ATP antibodies are used to capture the target analyte. Samples and standards are added sequentially, followed by HRP-conjugated detection antibody. After incubation and thorough washing, TMB substrate is added for color development. The color intensity, measured at 450 nm, is directly proportional to the ATP concentration in the sample. A standard curve is generated using serially diluted standards, and sample concentrations are calculated by interpolation from the standard curve.
Species	Human
Source	ELISA
Description	Principle: The kit uses a double-antibody one-step sandwich enzyme-linked immunosorbent assay (ELISA). Pre-coated microplate wells with ATP antibodies are sequentially loaded with specimens, standards, and HRP-labeled detection antibody, followed by incubation and thorough washing. TMB substrate is used for color development. TMB is converted to blue under the catalysis of peroxidase and then to the final yellow color under the action of acid. The depth of color is positively correlated with the ATP content in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader, and the sample concentration is calculated by interpolation from the standard curve. Intended Use: For research use

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only. Not for diagnostic or clinical use. Sample Types: Serum, plasma (EDTA, citrate, or heparin anticoagulant), cell culture supernatant, and tissue homogenate. Assay Range: 0 - 6400 nmol/L (Standard curve range: 0, 400, 800, 1600, 3200, 6400 nmol/L). Sensitivity: The minimum detectable concentration is less than 1.0 nmol/L. Specificity: No cross-reactivity with other soluble structural analogs. Precision: Intra-assay and inter-assay coefficients of variation are both less than 15%. Kit Components (96-well configuration): - Microplate (pre-coated): 12 wells × 8 strips - Standards (S0-S5): 0.3 mL × 6 tubes (concentrations: 0, 400, 800, 1600, 3200, 6400 nmol/L) - Sample Diluent: 6 mL - Detection Antibody-HRP: 10 mL - 20× Wash Buffer: 25 mL (dilute 1:20 with distilled water before use) - Substrate A: 6 mL - Substrate B: 6 mL - Stop Solution: 6 mL - Plate Sealer: 2 sheets - Self-sealing Bag: 1 piece Kit Components (48-well configuration): - Microplate (pre-coated): 12 wells × 4 strips - Standards (S0-S5): 0.3 mL × 6 tubes (concentrations: 0, 400, 800, 1600, 3200, 6400 nmol/L) - Sample Diluent: 3 mL - Detection Antibody-HRP: 5 mL - 20× Wash Buffer: 15 mL (dilute 1:20 with distilled water before use) - Substrate A: 3 mL - Substrate B: 3 mL - Stop Solution: 3 mL - Plate Sealer: 2 sheets - Self-sealing Bag: 1 piece Materials Required but Not Provided: 1. Microplate reader (450 nm wavelength) 2. High-precision pipettes and tips: 0.5-10 µL, 2-20 µL, 20-200 µL, 200-1000 µL 3. 37°C incubator or water bath Sample Collection, Processing, and Storage: 1. Serum: Use tubes without pyrogen and endotoxin. Avoid any cell stimulation during operation. After blood collection, centrifuge at 3000 rpm for 10 minutes to separate serum from red blood cells carefully and rapidly. 2. Plasma: Use EDTA, citrate, or heparin as anticoagulant. Centrifuge at 3000 rpm for 30 minutes and collect the supernatant. 3. Cell Culture Supernatant: Centrifuge at 3000 rpm for 10 minutes to remove particles and polymers. 4. Tissue Homogenate: Add appropriate amount of physiological saline to the tissue and homogenize. Centrifuge at 3000 rpm for 10 minutes and collect the supernatant. 5. Storage: If samples cannot be tested immediately, aliquot according to single-use amount and store at -20°C. Avoid repeated freeze-thaw cycles. Thaw at room temperature and ensure samples are uniformly and fully thawed before use.

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Reagent Preparation: Dilution of 20× Wash Buffer: Dilute with distilled water at a ratio of 1:20, i.e., 1 part of 20× wash buffer plus 19 parts of distilled water. Plate Washing Method: 1. Manual washing: Discard the liquid in the wells, fill each well with wash buffer, let stand for 1 minute, then discard the liquid and pat dry on absorbent paper. Repeat this washing process 5 times. 2. Automatic plate washer: Inject 350 µL of wash buffer into each well, soak for 1 minute, and wash 5 times. Assay Procedure: 1. Remove the required number of strips from the aluminum foil bag after equilibration at room temperature for 20 minutes. Return the remaining strips to the self-sealing bag and seal for storage at 4°C. 2. Set up standard wells and sample wells. Add 50 µL of different concentrations of standards to each standard well. 3. For sample wells, first add 10 µL of the sample to be tested, then add 40 µL of sample diluent. Do not add anything to blank wells. 4. Except for blank wells, add 100 µL of HRP-labeled detection antibody to each standard well and sample well. Seal the reaction wells with plate sealers and incubate at 37°C in a water bath or incubator for 60 minutes. 5. Discard the liquid, pat dry on absorbent paper, fill each well with wash buffer, let stand for 1 minute, discard the wash buffer, and pat dry on absorbent paper. Repeat this washing process 5 times (automatic plate washer can also be used). 6. Add 50 µL each of Substrate A and Substrate B to each well, and incubate at 37°C protected from light for 15 minutes. 7. Add 50 µL of stop solution to each well. Within 15 minutes, measure the OD value of each well at 450 nm wavelength. Result Calculation: Draw a standard curve: In an Excel worksheet, use the standard concentration as the X-axis and the corresponding OD value as the Y-axis to plot the standard linear regression curve. Calculate each sample concentration value according to the curve equation. Note: When following the instructions, the sample has been diluted 5-fold. The final result should be multiplied by 5 to obtain the actual sample concentration. Storage and Stability: Store the kit at 2-8°C, protected from light and moisture. The kit is stable for 6 months when stored properly. Cautions: 1. Store the kit at 2-8°C. Allow the kit to equilibrate at room temperature for 20 minutes before use. Crystals may form in the concentrated wash buffer when removed from

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	the refrigerator; this is normal. Heat in a water bath to dissolve crystals completely before use. 2. Unused plate strips should be immediately returned to the self-sealing bag and stored sealed (low temperature and dry). 3. The S0 standard with concentration 0 can be regarded as a negative control or blank. 4. Strictly follow the incubation times, reagent volumes, and sequence specified in the instructions. 5. Shake all liquid components well before use.
Form	ELISA Kit (96-well/48-well microplate format)
Applications	Quantitative detection of ATP in human serum, plasma, cell culture supernatant, and tissue homogenate samples. Suitable for research use in studies related to cell metabolism, energy status, cell viability, and proliferation.
Notes	1. Store the kit at 2-8°C. Allow the kit to equilibrate at room temperature for 20 minutes before use. Crystals may form in the concentrated wash buffer when removed from the refrigerator; this is normal. Heat in a water bath to dissolve crystals completely before use. 2. Unused plate strips should be immediately returned to the self-sealing bag and stored sealed (low temperature and dry). 3. The S0 standard with concentration 0 can be regarded as a negative control or blank; when following the instructions, the sample has been diluted 5-fold, and the final result should be multiplied by 5 to obtain the actual sample concentration. 4. Strictly follow the incubation times, reagent volumes, and sequence specified in the instructions. 5. Shake all liquid components well before use.
Storage	2-8°C, protected from light and moisture. Valid for 6 months.
Concentration	Standard concentrations (S0-S5): 0, 400, 800, 1600, 3200, 6400 nmol/L
Shipping	Shipped at ambient temperature. Upon receipt, store at 2-8°C.

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Reconstitution	20× Wash Buffer: Dilute 1:20 with distilled water (1 part 20× wash buffer + 19 parts distilled water).
Usage	For research use only. Not for diagnostic or clinical use.

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