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Human Anatabine ELISA Kit (Quantitative)

Product Number: ELK11694

Shipping and Storage

1. Kit storage temperature: 2-8 °C.
2. Shelf-life: 6 months.

Component

Component	96T
30-fold Concentrated Wash Buffer	20mL
HRP-Conjugated Reagent	6mL
Coated Microplate	12 wells × 8 strips
Chromogen solution A	6mL
Chromogen solution B	6mL
Stop solution	6mL
Sample diluent	6mL
Standard S1 (16ng/mL)	0.5mL
Standard S2 (8ng/mL)	0.5mL
Standard S3 (4ng/mL)	0.5mL
Standard S4 (2ng/mL)	0.5mL
Standard S5 (1ng/mL)	0.5mL

Description

This kit adopts competitive enzyme-linked immunosorbent assay for measuring Anatabine levels in specimens. Purified anti-Anatabine antibody is coated onto microplate wells to prepare solid-phase antibody. Anatabine sample and HRP-conjugated Anatabine antigen are added into antibody-coated wells for competitive binding. After thorough washing, chromogenic substrate TMB is added for color development. Color intensity is **negatively correlated** with Anatabine concentration in samples. Absorbance (OD value) is read by microplate reader at 450 nm wavelength, and Anatabine concentration in samples is calculated against the standard curve.

Application

This kit is used for the quantitative determination of Anatabine concentration in samples.

Detection Range

0.3ng/mL-18ng/mL

Specimen Requirements

1. Specimen treatment: Water sample: After collection, subject the sample to three repeated freeze- thaw cycles, then filter with glass- fiber filter for subsequent assay; Tissue sample: Extract samples with butanol- methanol- water (5 : 25 : 70, V : V : V), or perform extraction according to relevant literature protocols. Perform assay immediately after extraction. If immediate testing is unavailable, specimens may be stored at −20 °C for later testing.
2. Samples containing sodium azide (NaN_3) cannot be assayed, since NaN_3 inhibits horseradish peroxidase (HRP) activity.

Protocol

1. Sample Loading: Set standard wells, blank wells (no sample nor HRP-conjugated reagent added; all other steps remain

identical), and test sample wells. Add 50μL standard solution accurately into standard wells. For test sample wells, add 40μL Sample Diluent first, then add 10μL test sample (final sample dilution factor = 5). Dispense reagents to the bottom of microplate wells; avoid touching well side-walls. Mix gently by shaking.

2. Enzyme Addition: Add 50μL HRP-Conjugated Reagent to each well, excluding blank wells.
3. Incubation: Seal plate with plate-sealing film and incubate at 37℃ for 60 min.
4. Buffer Preparation: Dilute 30-fold Concentrated Wash Buffer 30-fold with distilled water for later use.
5. Washing: Carefully remove plate-sealing film, discard liquid and tap dry. Fill each well completely with wash buffer, hold for 30 s then discard. Repeat 5 times total, tap plate dry.
6. Color Development: Add 50μL Chromogenic Reagent A followed by 50μL Chromogenic Reagent B to each well. Mix by gentle shaking. Incubate at 37℃ in dark for 15 min for color development.
7. Termination: Add 50μL Stop Solution into each well to terminate reaction (blue color immediately turns yellow).
8. Measurement: Zero instrument against blank wells. Read absorbance (OD value) of each well sequentially at 450nm. Complete measurement within 15 min after adding Stop Solution.

Calculation

Plot standard curve on graph paper with standard concentration as X-axis and corresponding OD value as Y-axis. Read corresponding concentration from standard curve according to sample OD value and multiply by dilution factor.

Alternatively, establish regression equation of standard curve using standard concentrations and OD values. Substitute sample OD value into the equation to calculate sample concentration, then multiply by dilution factor to obtain actual sample concentration.

Note

1. Equilibrate kit to room temperature for 15-30 min after removing from refrigerated storage before use. For unused strips after opening coated microplate, store strips inside the sealing bag.
2. Crystals may form in concentrated wash buffer. Warm in water-bath to dissolve crystals during dilution; crystal dissolution will not affect washing performance or assay results.
3. Use pipettors for all dispensing steps; calibrate pipettors regularly to reduce experimental error. Complete all sample loading within 5 min. For large-batch specimens, multichannel pipettors are recommended.
4. Run standard curve in parallel for every assay; duplicate wells are preferred. If analyte concentration in specimen is excessively high (sample OD lower than OD of highest-concentration standard well), pre-dilute sample n-fold with Sample Diluent before measurement. Multiply final result by total dilution factor (× n × 5) in calculation.
5. Plate-sealing films are for single-use only to avoid cross-contamination.
6. Store chromogenic substrate away from light.
7. Strictly follow manual procedures. Assay results must be determined from microplate-reader readings.
8. Treat all samples, wash buffers and waste materials as infectious biohazardous waste.
9. Components from different kit batch numbers shall not be mixed.