Atenolol ELISA Kit

Catalog Number KA6305

96 assays

Version: 01

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of atenolol in sample.

Background

Atenolol, 4-(2-hydroxy-3-isopropyl-aminopropoxy)-phenylacetamide, is a β_1 selective adrenoceptor antagonist used in the treatment of angina and hypertension. Atenolol is poorly protein bound. Plasma half-life is 6-8 hours but can rise to very long hours in severe renal impairment. There is no liver metabolism, 90 % of absorbed drug is excreted unchanged in the urine.

Principle of the Assay

The enzyme immunoassay for Atenolol is based on the competition between the Atenolol in the sample and the Atenolol-horseradish peroxidase conjugate, for binding to rabbit antibody directed against Atenolol, coated onto microwells. The sample containing the Atenolol, and the Atenolol-horseradish peroxidase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. If no or small amount of Atenolol is present in the sample more enzyme labeled Atenolol will bind the antibody on the solid surface. On the other hand, if large or significant amount of Atenolol is present in urine sample, less enzyme labeled Atenolol will bind to the antibody, producing less color signal. Therefore, the intensity of the color developed is inversely proportional to the concentration of Atenolol in the sample. The concentration is calculated on the basis of a standard curve.

General Information

Materials Supplied

List of component

Component	Amount
96-wells microtiter plate (#S). Twelve strips of 8 detachable wells, coated with Anti-Atenolol antibody.	96 (8x12) wells
Calibrators containing 0, 1, 7.5 and 60 ng/mL of Atenolol	0.6 mL x 4
Atenolol-Horseradish Peroxidase Conjugate (ATL-HRP) (#3)	10.5 mL
Stabilized tetramethylbenzidine (TMB) substrate. Ready to use. (#5)	10.5 mL
Wash Buffer (10xPBS-Tween). Dilute 10 fold with distilled or deionized water to 150 mL prior to use. (#6)	15 mL
Stop Solution, 3 N HCl. (#7)	10.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8 °C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipetters capable of delivering 25 µL, 50 µL and 100 µL.
- ✓ Microtiter plate reader (wavelength 450 nm).
- ✓ Plate washer or squeezable wash bottle.
- ✓ Timer.
- ✓ Absorbent paper towels.

Precautions for Use

Reagents are for in vitro research use only.

- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of atenolol in the samples are high, dilute sample such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains HCl. Avoid contact with skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 25 µL of standard or sample to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of ATL-HRP conjugate **(#3)** to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 30 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of TMB substrate (#5) to each well and incubate at room temperature for 15 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 100 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 450 nm using an ELISA reader.

✓ Simplified Assay Procedure

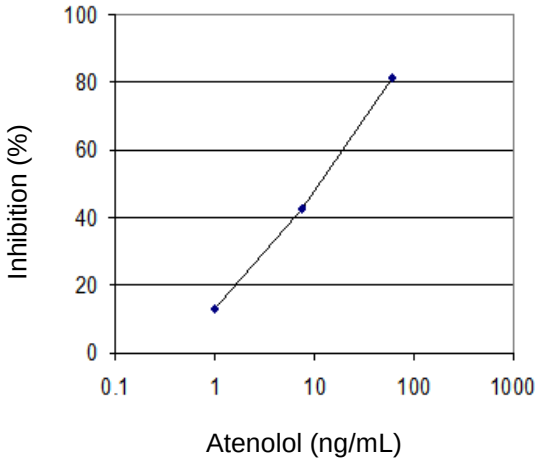
1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 30 min at RT.
3. Wash 3x.
4. Add TMB substrate (100 µL), wait for 15 min. at RT.
5. Add stopping solution (100 µL) and read at 450 nm.

Data Analysis

Calculation of Results

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of atenolol including 0 ng/mL (OD_0).
   (b) % of Inhibition = 100 - (OD_s / OD_0) x 100
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate atenolol concentration of sample by interpolation and multiply by dilution factor to obtain the actual quantity of atenolol.

✓ Atenolol Inhibition curve



Resources

Plate Layout

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