FK-506 ELISA Kit

Catalog Number KA1417

96 assays

Version: 100

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of FK-506 in Sample Extract.

Background

FK-506 (Tacrolimus) is a microlide lactone of fugal origin with potent immunosuppressive properties. FK-506 is used for primary or rescue immunosuppressant in patients after solid organ transplantation. Clinically relevant FK-506 side effects are nephrotoxicity, neurotoxicity, diabetes mellitus and hypertension. Most of these side effects are more closely related to FK-506 blood levels than dosage, therefore therapeutic drug monitoring of FK-506 levels is a prerequisite for therapy.

Principle of the Assay

The enzyme immunoassay for FK-506 ELISA Kit is based on the competition between the FK-506 to be assayed and the FK-506-Horseradish Peroxidase conjugate, for binding to rabbit antibody directed against FK-506, coated onto microwells. The sample containing the FK-506, and the FK-506-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. The intensity of the color developed is inversely proportional to the concentration of FK-506 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: Twelve strips of 8 detachable wells, coated with Anti-FK-506 antibody (#S).	96 (12 x 8) wells
Calibrator: Containing 0, 2, 10 and 50 ng/mL of FK-506.	0.6 mL x 4
FK-506-Horseradish Peroxidase conjugate (ACC-HRP) (#3).	10.5 mL
Stabilized Tetramethylbenzidine (TMB) substrate. Ready to use (#5).	10.5 mL
Wash Buffer (10x PBS-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

- ✓ Pipettors 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

Reagent are for in vitro research use only.

- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of FK-506 in the samples are high (>100 ng/mL), 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 acid. 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 samples 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 ACC-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

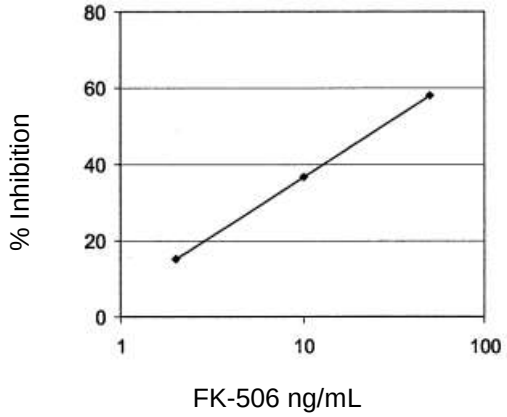
1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 30 min at RT.
3. Wash 3x.
4. Add TMB (100 µL), wait for 15 min. at RT.
5. Add Stop Solution (100 µL) and read at 450 nm.

Data Analysis

Calculation of Results

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of FK-506 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 FK-506 concentration of sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of FK-506.

✓ FK-506 Inhibition curve



Performance Characteristics

✓ Cross Reactivity

Except Ascomycin, some other compounds tested at the stated levels are found to give results not greater than a 2.5 ng/mL of FK-506.

Compound	Conc (ng/mL)	% Inhibition
Ascomycin	10	38%
Gentamicin	10,000	<10
G-418	10,000	<10
Monensin	10,000	<10
Sulfamethazine	10,000	<10
Sulfadimethoxine	10,000	<10
Zearalenone	10,000	<10
Narasin	10,000	<10

There is the possibility that other substances and/or factors not listed above may interfere with the test.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H