Paraquat ELISA Kit

Catalog Number KA1424

96 assays

Version: 57

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the determination of Paraquat in Samples.

Background

The herbicide paraquat (1,1'-dimethyl-4,4'-bipuridinium ion) is a potent herbicide, useful for the control of both terrestrial and aquatic weeds. It was also used as preharvest desiccant and defoliant. The use for these purposes requires a sensitive method for the estimation of residues in various crops, soil and water. Estimation of the concentrations of paraquat in biological fluids is a useful diagnostic and in cases of poisoning. It has been shown that paraquat concentrations in human plasma can be estimated by enzyme immunoassay and that the results so obtained correlate well with those of other methods.

Principle of the Assay

The enzyme immunoassay for paraquat is based on the competition between the paraquat in the sample and the Paraquat-Horseradish Peroxidase conjugate, for binding to antibody directed against paraquat, coated onto microwells. The sample containing the paraquat, and the Paraquat-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 paraquat is present in the sample more enzyme labeled paraquat will bind the antibody on the solid surface. On the other hand, if large or significant amount of paraquat is present in urine sample, less enzyme labeled paraquat will bind to the antibody, producing less color signal. Therefore, the intensity of the color developed is inversely proportional to the concentration of paraquat 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-Paraquat antibody.	96 (8x12) wells
Calibrators: Containing of 0, 0.75, 2.5 and 7.5 ng/mL of Paraquat.	0.6 mL x 4
Paraquat-Horseradish Peroxidase Conjugate (PRQ-HRP) (#3)	10.5 mL
Stabilized tetramethylbenzidine (TMB) substrate (#5): Ready to use.	10.5 mL
Wash Buffer (10x PBS-Tween) (#6): Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution (#7): 3 N HCl.	10.5 mL

Storage Instruction

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

Materials Required but Not Supplied

- ✓ Pipetters capable of delivering 25 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.

- ✓ Store reagents at 2 to 8 °C, and do not use beyond expiration date. Never freeze kit components.
- ✓ Do not return unused reagents back into their original bottles. The assay procedure details volumes required.
- ✓ 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. Do not allow to contact skin or eyes. If exposed, flush with water.
- ✓ Consider all materials, containers and devices that are exposed to sample or standards to be contaminated with toxin. Wear protective gloves and safely glasses when using this kit.
- ✓ 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 PRQ-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 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

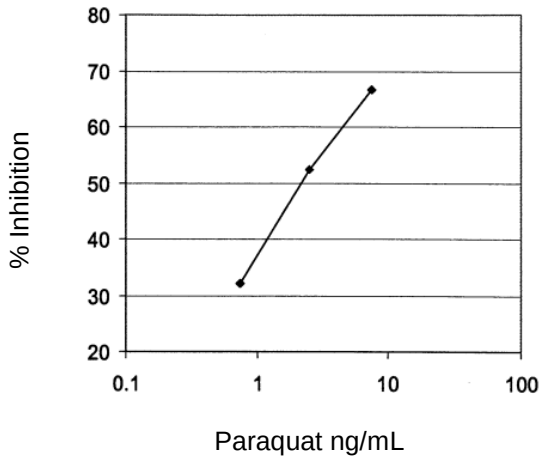
1. Add sample or standard (25 µL).
2. Add enzyme conjugate (100 µL). 30 min at RT.
3. Wash 3x.
4. Add TMB (100 µL), wait for 15 min. at RT.
5. Add stop solution (100 µL) and read at 450 nm.

Data Analysis

Calculation of Results

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

✓ Paraquat Inhibition curve



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