Abraxis 2,4-D Plate Kit

Cat. # 54003B

Instructional Booklet
Read Completely Before Use.

INTENDED USE

The Abraxis 2,4-D Plate Kit is a competitive ELISA for the quantitative analysis of 2,4-D in water samples with the range of 2-200 ppb. Samples containing higher concentration can be measured by diluting the sample.

ASSAY PRINCIPLES

The Abraxis 2,4-D plate kit is a competitive enzyme-labeled immunoassay. 2,4-D calibrators and sample(s) are pipetted into the test wells followed by 2,4-D HRP conjugate into the test wells to initiate the reaction. During the 60 minute incubation period, 2,4-D from the sample and 2,4-D HRP conjugate compete for binding to 2,4-D antibody bound to plastic wells on the microtiter plate. Following this 60 minute incubation, the contents of the well are removed and the wells are washed to remove any unbound 2,4-D, 2,4-D HRP conjugate. After washing with wash solution, a clear substrate is then added to the wells and any bound enzyme conjugate causes the conversion to a blue color. Following a30 minute incubation, the reaction is stopped and the amount of color in each well is read. The color of the unknown samples is compared to the color of the calibrators and the 2,4-D concentration of the samples is derived.

SPECIFICITY:

The Abraxis 2,4-D Plate Kit can not differentiate between the various 2,4-Ds, but detects their presence to differing degrees. The following table shows the % cross reactivity of the antibody. All concentrations are in parts per billion (ppb).

Compound	% CR
2,4-D	**100%**
2,4-D methyl ester	**400%**
2,4-DB	**100%**
2,4-D isopropyl ester	**67%**
2,4-DB-butyl ester	**53**
2,4,5-T	**9.5**
MCPA	**9.3**
Dichlorprop	**2.7**
2,4,5-TP	**2.2**

REAGENTS AND MATERIALS PROVIDED

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8ºC.

- Plate containing 12 test strips of 8 wells coated with anti 2,4-D, each vacuum-packed in aluminized pouch with indicating dessicant.
- 1 vials each containing a 2,4-D calibrators ready to use at 0, 2, 20 and 200 ppb.
- 1 vial containing 7 mL 2,4-D HRP Enzyme Conjugate.
- 1 bottle containing (5X) Wash solution. Please dilute before use, 100 mL of Wash solution (5X) and 400 mL of DI water. **(NOTE:** Please use the 2,4-D Wash Solution only with 2,4-D Test Kit, this solution has been formulated specially for this ELISA kit)
- 1 vial containing 14 mL of Substrate (Color Solution)
- 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- Instructions

PRECAUTIONS

1. Each reagent is optimized for use in the Abraxis 2,4-D Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Abraxis 2,4-D Plate Kits with different Lot numbers.

2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.

3. Do not use reagents after expiration date.

4. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.

5. 2,4-D should be treated with care.

6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Laboratory quality distilled or deionized water.

2. Graduated cylinder, 100 ml or larger.

3. Glassware for standard dilutions.

4. Pipet with disposable tips capable of dispensing 50 µL.
5. Multi-channel pipet; 8 channel capable of dispensing 50 and 100 µL.
6. Paper towels or equivalent absorbent material.
7. Microwell plate or strip reader with 450nm filter.
8. Timer
9. Vortex mixer
10. Wash bottle

TEST PROCEDURE (Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of test wells and into a microwell holder. Be sure to re-seal unused wells in the zip-lock bag with dessicant.
3. Using a pipet with disposable tips, add **50 ul of Calibrators or Sample in duplicate** to the appropriate test wells. Be sure to use a clean pipet tip for each. Add **50 µL enzyme conjugate** to corresponding well.
5. Rotate the plate gently for 30 seconds and incubate the test wells for **60 minutes**.
7. Dump the contents of the wells into an appropriate waste container. Pipette 250 uL of (1X) wash Solution and dump (blot on paper towels between washes). Repeat 4X for a total of five washes.
8. Following the last wash tap the inverted wells onto absorbent paper to remove the last of the wash solution.
9. Dispense **100 µL of Substrate** into each well.
10. Incubate the wells for **30 minutes**.
11. Dispense **100 µL of Stop Solution** into each test well.
12. Read and record the absorbance of the wells at 450nm using a strip or plate reader.

RESULTS INTERPRETATION

1. Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells: Sample containing less color than a calibrator well have a concentration of 2,4-D greater than the concentration of the calibrator. Samples containing more color than a calibrator well have a concentration less than the concentration of the calibrator.

Quantitative Interpretation

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

   %Bo = (average OD of calibrator or sample x 100) ÷ average OD of negative control

2. Graph the %Bo of each calibrator on the Y (linear) axis against its 2,4-D concentration on the X (log) axis using semi-log graph paper. Draw the best fit line through the calibrator points.

3. Determine the 2,4-D concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.

Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Alternatively, Abraxis can supply a 4-paremeter spreadsheet template which can be used for data reduction. Please contact Abraxis for further details.

GENERAL LIMITED WARRANTY

Abraxis LLC ("Abraxis") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. ABRAXIS MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Abraxis products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Abraxis. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Abraxis's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Abraxis promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Abraxis is willing and able to repair or replace any nonconforming Abraxis product or part. Abraxis shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

ABRAXIS LLC
54 Steamwhistle Drive
Warminster, PA 18974

Tel. (215) 357-3911
Fax (215) 357-5232
www.Abraxiskits.com

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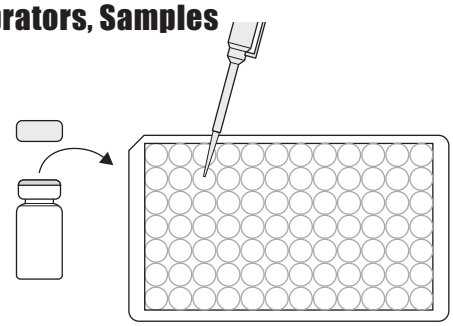
2,4-D Plate, Detailed ELISA Procedure

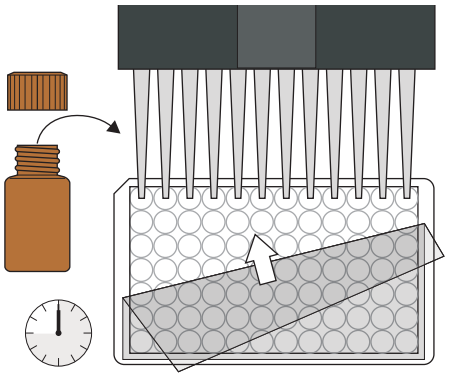
1. Addition of Calibrators, Samples

Add 50 uL of calibrators or sample into the wells of the test strips according to the working scheme given. Be sure to use a clean pipet tip for each.



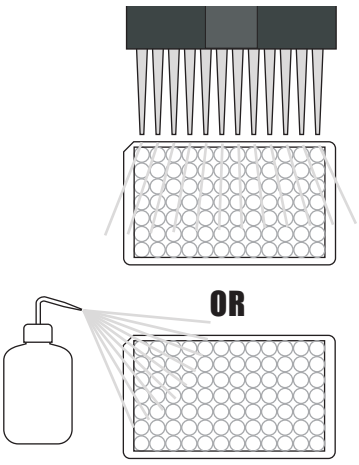
2. Addition of Enzyme Conjugate

Add 50 uL of the Enzyme Conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 60 seconds. Be careful not to spill contents. Incubate the strips for 60 min at room temperature.



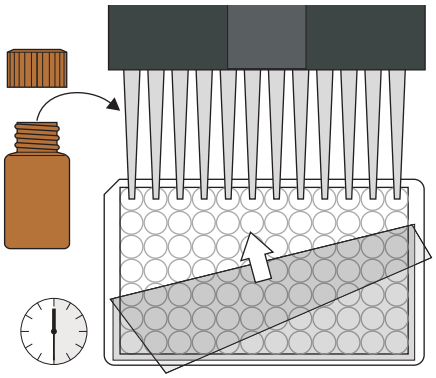
3. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips five times with a multi-channel pipette or wash bottle using the diluted 5X washing buffer solution. Please use at least a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



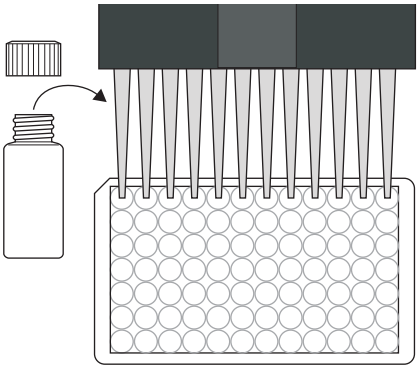
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



5. Addition of Stopping Solution

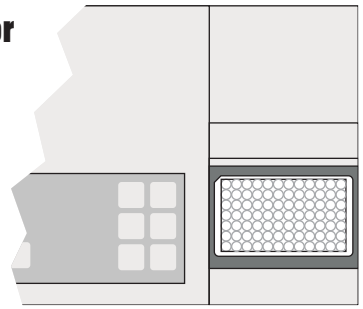
Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



6. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

NOTE: Multiply ELISA results by appropriate dilution factors.

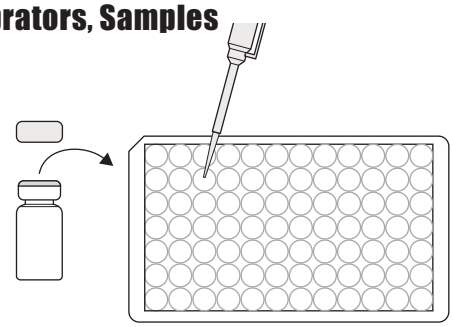


For Ordering or Technical Assistance Contact:
ABRAXIS, LLC
54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

2,4-D Plate, Concise ELISA Procedure

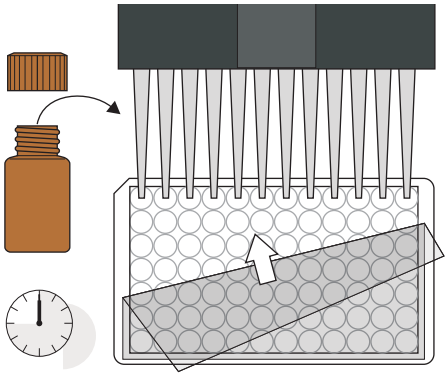
1. Addition of Calibrators, Samples

Add 50 uL of calibrators or diluted sample extracts.



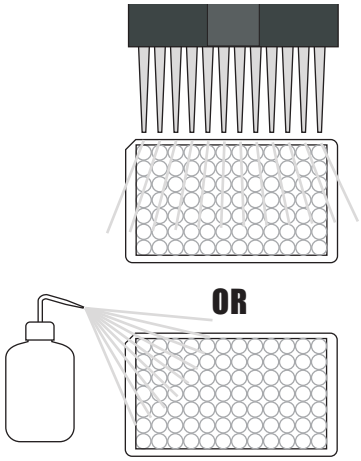
2. Addition of Enzyme Conjugate

Add 50 uL of the Enzyme Conjugate. Cover and mix for 60 seconds by rotating on benchtop. Incubate for 60 minutes at room temperature.



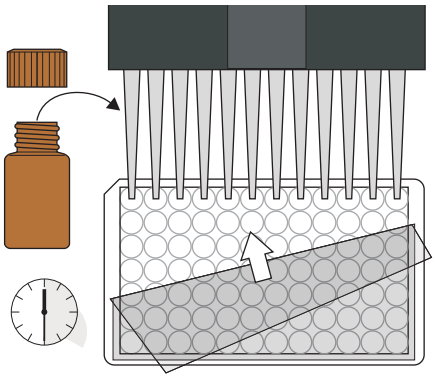
3. Washing of Plates

Wash the plates five times with 250 uL of diluted 1X washing buffer.



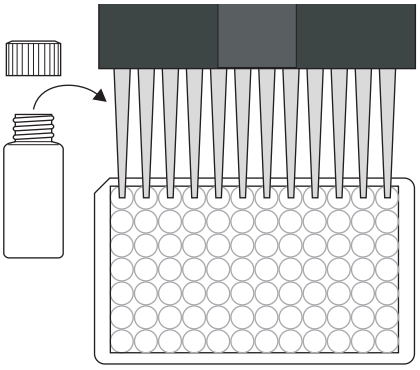
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 30 minutes at room temperature and away from direct sunlight.



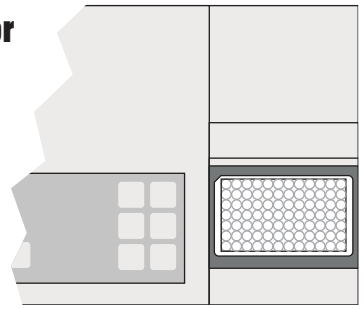
5. Addition of Stopping Solution

Add 100 uL of stop solution.



6. Measurement of Color

Measure color at 450 nm. Calculate results.



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