Paraquat

• Intended Use

For detection of free paraquat cation. Application procedures for various sample matrices (e.g., fruits and vegetables) can be obtained from Strategic Diagnostics Inc.

• Principle

The Paraquat RaPID Assay® applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of free paraquat cation. Samples are processed as described in individual application procedures. Aliquots of prepared samples are then diluted in a sample diluent. The diluted sample to be tested is added, along with enzyme conjugate, to a disposable test ube, followed by paramagnetic particles with antibodies specific to paraquat attached. Both the paraquat (which may be in the sample) and the enzyme labeled paraquat (in the enzyme conjugate) compete for antibody sites on the magnetic particles. At the end of the incubation period, a magnetic field is applied to hold the paramagnetic particles (with paraquat and labeled paraquat analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of paraquat is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled paraquat analog bound to the paraquat antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of acid. Since the labeled paraquat (conjugate) was in competition with the unlabeled paraquat (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of paraquat in the sample** .

• Reagents

1. Paraquat Antibody Coupled Paramagnetic Particles
The paraquat antibody (rabbit anti-paraquat) is covalently bound to paramagnetic particles, which are suspended in buffered saline with preservative and stabilizers.
30 test kit: one 20 mL vial
100 test kit: one 65 mL vial

2. Paraquat Enzyme Conjugate
The horseradish peroxidase (HRP) labeled paraquat analog is diluted in buffered saline containing preservative and stabilizers.
30 test kit: one 10 mL vial
100 test kit: one 35 mL vial

3. Paraquat Standards
Three concentrations (50, 250, 500 ppt) of paraquat cation standards in buffered saline with preservative and stabilizers are supplied. Each vial contains 2.0 mL.

4. Control
A concentration (approximately 100 ppt) of paraquat cation in buffered saline containing preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
Buffered saline containing preservative and stabilizers without any detectable paraquat.
30 test kit: one 10 mL vial
one 100 mL vial
100 test kit: one 35 mL vial
three 100 mL vials

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
30 test kit: one 20 mL vial
100 test kit: one 65 mL vial

7. Stopping Solution
A solution of sulfuric acid (0.5%).
30 test kit: one 20 mL vial
100 test kit: one 60 mL vial

8. Washing Solution
Preserved deionized water.
30 test kit: one 70 mL vial
100 test kit: one 250 mL vial

9. Test Tubes
Polystyrene tubes (36) are packaged in a box.
30 test kit: one 36 tube box
100 test kit: three 36 tube boxes

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets* Precision pipets capable of delivering 200, 250 and 500 uL and a 1.0 mL repeating pipet.

Vortex Mixer* Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation Rack*

RPA-I™ RaPID Analyzer* or equivalent photometer capable of readings at 450 nm

* These items are available from Strategic Diagnostics Inc.

• Sample Information

Refer to sample preparation information contained under individual procedure or application note.

Analysis of paraquat is complicated by its ionic nature. Paraquat, which exists as a charged cationic species in solution, is prone to strong adsorption to organic and inorganic adsorbents. **All sources of adsorption, i.e., glassware, should be avoided to prevent loss of analyte. It is recommended that samples be collected and stored in plastic vessels (e.g. polypropylene).**

If the paraquat concentration of a sample exceeds 500 ppt, the sample is subject to repeat testing using an increased sample dilution in the Diluent/Zero Standard or Sample Diluent. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the corrected dilution factor.

• Reagent Preparation

All reagents must be allowed to come to room temperature and the antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation rack consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes**.

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube (technique is demonstrated on training video, available from Strategic Diagnostics Inc.).

Mix the antibody coupled paramagnetic particles just prior to pipetting.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Paraquat RaPID Assay will detect paraquat cation and related compounds to different degrees. Refer to specificity table for data. The Paraquat RaPID Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 100 ppt of paraquat cation is provided with the Paraquat RaPID Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Perform the appropriate sample preparation (refer to specific application procedure).
2. Label test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppt
3,4	Standard 1, 50 ppt
5,6	Standard 2, 250 ppt
7,8	Standard 3, 500 ppt
9	Control
10	Sample 1
11	Sample 2

3. Add 200 uL of the appropriate standard, control, or sample.
4. Add 250 uL of Paraquat Enzyme Conjugate to each tube.
5. Mix the Paraquat Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
6. Vortex for 1 to 2 seconds minimizing foaming.
7. Incubate for 15 minutes at room temperature.
8. Separate in the Magnetic Separation Rack for **two (2) minutes**.
9. Decant and **gently** blot all tubes briefly in a consistent manner.
10. Add 1 mL of Washing Solution to each tube and allow them to remain in the magnetic separation unit for **two (2) minutes**.
11. Decant and **gently** blot all tubes briefly in a consistent manner.
12. Repeat Steps 10 and 11 an additional time.
13. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
14. Vortex for 1 to 2 seconds minimizing foaming.
15. Incubate for 20 minutes at room temperature.
16. Add 500 uL of Stopping Solution to each tube.
17. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 18.
18. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding paraquat cation concentration on horizontal logarithmic (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppt of paraquat by interpolation using the standard curve.
5. Determine paraquat concentration in sample by multipying result by appropriate dilution and sample preparation factors (see application proceudre).

(Contact SDI for detailed application information on specific photometers.)

RPA-I RaPID Analyzer

Using the RPA-I RaPID Analyzer, calibration curves can be automatically calculated and stored. Refer to the RPA-I operating manual for detailed instructions. To obtain results from the Paraquat RaPID Assay on the RPA-I the following parameter settings are recommended:

Data Reduct	:	Lin. Regression
Xformation	:	Ln/LogitB
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPT
# Rgt Blk	:	0

Calibrators:

# of Cals	:	4
# of Reps	:	2

Concentrations:

#1:	00.0	PPT
#2:	50.0	PPT
#3:	250.0	PPT
#4:	500.0	PPT

Range	:	20 - 500
Correlation	:	0.990
Rep. %CV	:	10%

Conversion Factors

The Paraquat RaPID Assay is calibrated to paraquat cation concentrations. To convert to equivalent concentrations of paraquat dichloride use the following formulas:

paraquat cation x 1.38 = paraquat dichloride
paraquat dichloride x 0.72 = paraquat cation

• Expected Results

Refer to the expected result section in the appropriate application note or procedure.

• Performance Data

Precision

The following results were obtained using various paraquat samples prepared in the Diluent/Zero Standard:

Control	1	2	3	4
Replicates	5	5	5	5
Days	5	5	5	5
n	25	25	25	25
Mean (ppt)	45.46	106.1	225.5	427.7
% CV (within assay)	17.2	14.4	7.5	7.0
% CV (between assay)	<0.1	11.8	<0.1	<0.1

Sensitivity

The Paraquat RaPID Assay has an estimated minimum detectable concentration in buffer , based on a 90% B/Bo of 20 ppt. Refer to appropriate application notes or procedure for least detectable dose in specific crops.

Specificity

The cross-reactivity of the Paraquat RaPID Assay for paraquat, other bipyridinium herbicides and metabolites can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)
Paraquat cation	0.02	0.30
Methylbipyridyl methly sulphonium salt	0.002	0.96
Diethyl paraquat	0.005	13
Monoquat	0.94	44
Morphamquat	13	746
Diquat	112	7502
4,4-Bipyridyl	1860	>10,000
Chlormequat	3300	>10,000
Allyl Trimethyl ammonium bromide	6600	>10,000
1-Methyl-4-carboxy pyridinium	7100	>10,000
(2-Bromoethyl) tri-methyl ammonium bromide	>10,000	>10,000
Methylamine hydrochloride	>10,000	>10,000
Pincolinic acid	>10,000	>10,000

The following compounds demonstrated no reactivity in the Paraquat RaPID Assay at concentrations up to 10 ppm: alachlor, aldicarb, aldicarb sulfoxide, aldicarb sulfone, ametryn, atrazine, butylate, carbaryl, carbofuran, cyanazine, 2,4-D, dicamba, dieldrin, dinoseb, iprodione, MCPA, mecoprop,metalxyl, methomyl, metolachlor, metribuzin, pentachlorophenol, picloram, phosphamidon, procymidone, prometon, prometryn, propachlor, propazine, silvex, simazine, terbufos, terbutryn, vinclozolin.

• Assistance

For ordering or technical assistance contact:

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111 Pencader Drive
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Phone(800)544-8881
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techservice@sdix.com

• Availability

Strategic Diagnostics Inc.
Paraquat RaPID Assay
100 Test Kit
Paraquat Sample Diluent

Z00154 R020498