

Picloram

• Intended Use

For detection of picloram in water (groundwater, surface water, well water). For soil and other matrices refer to specific application bulletins.

• Principle

The Picloram RaPID Assay[®] applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of picloram. The sample to be tested is added to a disposable test tube along with an enzyme conjugate, followed by paramagnetic particles with antibodies specific to picloram attached. Both the picloram (which may be in the sample) and the enzyme labeled picloram (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles (with picloram and labeled picloram 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 picloram is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled picloram analog bound to the picloram 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 picloram (conjugate) was in competition with the unlabeled picloram (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of picloram in the sample.**

• Reagents

1. Picloram Antibody Coupled Paramagnetic Particles

The picloram antibody (mouse anti-picloram) 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. Picloram Enzyme Conjugate

The horseradish peroxidase (HRP) labeled picloram analog is diluted in buffered saline containing preservatives and stabilizers.

30 test kit: one 10 mL vial
100 test kit: one 35 mL vial

3. Picloram Standards

Three concentrations (2.0, 8.0, 20.0 ppb) of picloram standards in buffered saline with preservative and stabilizers are supplied. Each vial contains 2.0 mL.

4. Control

A concentration (approximately 10.0 ppb) of picloram in buffered saline with 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 picloram.

30 test kit: one 10 mL vial
100 test kit: one 35 mL vial

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 (D)

Preserved deionized water with detergent.

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 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 250 and 500 uL and a 1.0 mL repeating pipette.

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

Magnetic Separation Rack*

RPA-ITM[™] RaPID Analyzer* or equivalent photometer capable of readings at 450 nm

* These items are available from Strategic Diagnostics, Inc.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

If the picloram concentration of a sample exceeds 20.0 ppb, the sample is subject to repeat testing using a diluted sample. A five-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a five fold dilution by adding 200 uL of the sample to 800 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtained by the dilution factor, e.g., 5.

• 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 pipette tip**. This will help assure consistent quantities of reagent in the test mixture.

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

Avoid foam formation during vortexing.

Avoid performing steps 12-17 in direct sunlight.

Do not use any reagents beyond their stated shelf life.

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 (the technique is demonstrated on training video, available from Strategic Diagnostics, Inc.).

Mix the antibody coupled paramagnetic particles just prior to pipetting.

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 Picloram RaPID Assay will detect picloram and related compounds to different degrees. Refer to specificity table for data on several related compounds. The Picloram RaPID Assay kit provides screening results. As with any analytical technique (HPLC, GC, 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 10.0 ppb of picloram is provided with the Picloram 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. Label test tubes for standards, control and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 2.0 ppb
5,6	Standard 2, 8.0 ppb
7,8	Standard 3, 20.0 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

2. Add 250 uL of the appropriate standard, control, or sample.
3. Add 250 uL of Picloram Enzyme Conjugate to each tube.
4. Mix the Picloram Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 30 minutes at room temperature.
7. Separate in the Magnetic Separation Rack for **two (2) minutes**.

8. Decant and gently blot all tubes briefly in a consistent manner.	n	25	25	25	25
9. Add 1 mL of Washing Solution to each tube and allow them to remain in the magnetic separation unit for two (2) minutes .	Mean (ppb)	3.88	6.26	10.45	15.37
	% CV (within assay)	10.9	8.5	9.7	7.3
	% CV (between assay)	7.6	3.8	<0.1	1.6

Recovery

Four (4) samples, including tap water, a rainwater sample and a sample from a small creek were spiked with various levels of picloram and then assayed using the Picloram Assay. The following results were obtained:

Amount of Picloram Added (ppb)	Recovery		
	Mean (ppb)	S.D. (ppb)	%
4.0	3.76	0.60	94
6.0	5.94	0.46	99
10.0	9.89	0.44	99
15.0	15.3	0.90	102
Average			99

• Results

Manual Calculations

- Calculate the mean absorbance value for each of the standards.
- 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.
- Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding picloram concentration on horizontal logarithmic (X) axis on the graph paper provided.
- %B/Bo for controls and samples will then yield levels in ppb of picloram by interpolation using the standard curve.

(Contact Strategic Diagnostics, Inc. 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 Picloram 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 : PPB
 # Rgt Blk : 0

Calibrators:
 # of Cals : 4
 # of Reps : 2

Concentrations:
 #1: 0.00 PPB
 #2: 2.0 PPB
 #3: 8.0 PPB
 #4: 20.0 PPB

Range : 0.87 - 20.0
 Correlation : 0.990
 Rep. %CV : 10%

• Performance Data

Sensitivity

The Picloram Assay has an estimated minimum detectable concentration of 0.87 ppb, based on a 90% B/Bo.

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	5	5	5	5
Days	5	5	5	5

• Assistance

For ordering or technical assistance contact:
 Strategic Diagnostics Inc.
 111 Pencader Drive
 Newark, Delaware 19702-3322 USA
 800-544-8881
 Tel 302-456-6789
 Fax 302-456-6782
 techservice@sdx.com

• Availability

Strategic Diagnostics Inc.
 Picloram Assay
 100 Test Kit
 Picloram Proficiency Samples
 Picloram Sample Diluent

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