



*Scope and Application: For water*

\* This test is semi-quantitative. Results are expressed as greater or less than the threshold value used.

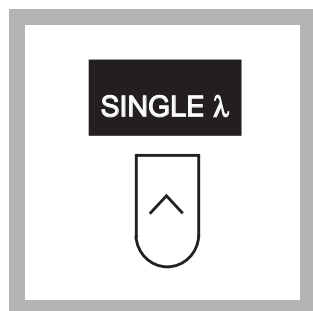
This method analyzes for Atrazine in water. Sample calibrators and reagents are added to cuvettes coated with Atrazine-specific antibodies. The color that develops is then measured and compared with the color measurements of the calibrators. The test requires about 30 minutes for complete analysis. As many as 20 cuvettes (18 samples and 2 calibrators) can be run simultaneously.

## **Tips and Techniques**

- **Read the entire procedure before starting.** Identify and have ready all the necessary reagents, cuvettes, and other apparatus before beginning the analysis.
- **Timing is critical;** follow instructions carefully.
- **A consistent technique when mixing the cuvettes is critical to this test.** The best results come from using the cuvette rack and mixing as described in *Using the 1-cm MicroCuvette Rack*. Cuvettes can be mixed individually, but test results may not be as consistent.
- Handle the cuvettes carefully. Scratches on the inside or outside may cause erroneous results. Carefully clean the outside of the cuvettes with a clean absorbent cloth or tissue before placing them into the instrument. (Kimwipe® tissues are provided with the kit.)
- Antibody cuvettes and enzyme conjugate are made in matched lots. Do not mix reagent lots.
- To avoid damaging the Color Developing Solution, do not expose it to direct sunlight.
- The cuvette rack is designed to be inverted with the cuvettes in place. This is especially helpful when running many samples at once; the cuvettes can remain in the rack and be processed together until they are read in the Immunoassay Pocket Colorimeter.
- Twenty Antibody Cuvettes are provided with each reagent set. One Antibody Cuvette will be used for each calibrator and each sample. Cuvettes are not reusable.
- Ensure the 1cm MicroCell adapter is installed in the DR/4000.

**Note:** Hach Company recommends wearing protective nitrile gloves for this procedure.

## Immunoassay



**1.** Press the soft key under **SINGLE λ**.

Press the soft key under **GO TO λ**.

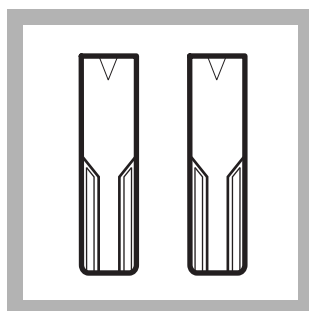
Select 450 nm by pressing the numeric keys **450**.

Press: **ENTER**

**Note:** The Flow Cell and Sipper Modules cannot be used with this procedure.

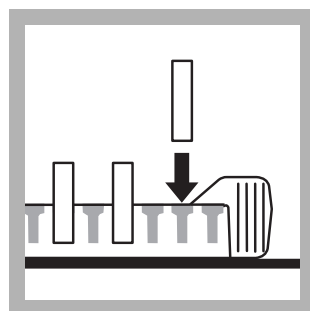


**2.** The display will show: **ZERO REQUIRED**

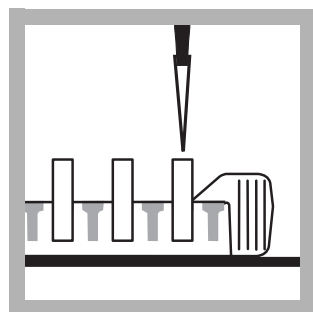


**3.** Label an Antibody Cuvette for each calibrator and each sample to be tested.

**Note:** As many as 20 cuvettes may be tested at one time and may comprise any combination of samples and calibrators.

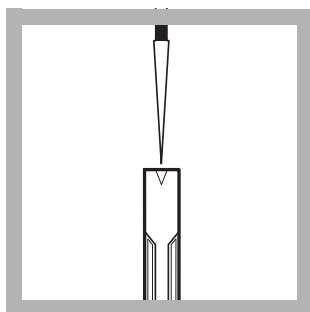


**4.** Place the cuvettes into the rack snugly.



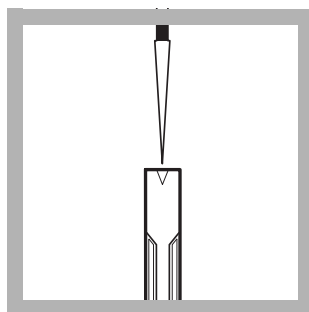
**5.** Pipet 0.5 mL of each calibrator into the appropriately labeled cuvette.

**Note:** Use a new pipette tip for each sample.

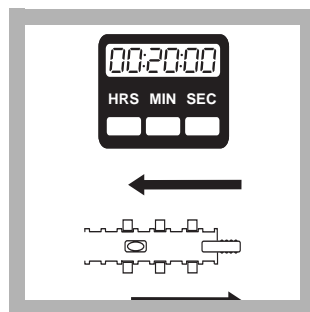


**6.** Pipet 0.5 mL of each sample to be tested into the appropriately labeled cuvette.

**Note:** Use a new pipette tip for each sample.



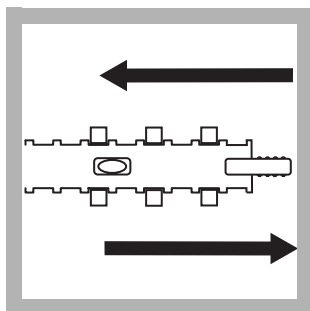
**7.** Immediately pipet 0.5 mL of Atrazine Enzyme Conjugate into each cuvette.



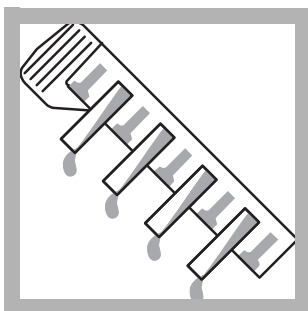
**8.** Key **2000**. Press the soft key under **START TIMER**.

A 20-minute reaction time will begin.

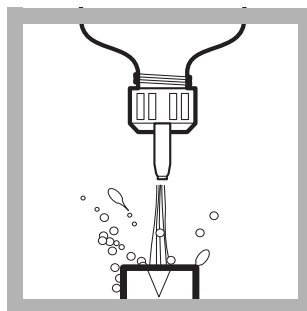
Immediately mix the contents of the cuvettes for 30 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.



**9.** After 10 minutes mix the contents of the rack for 30 seconds using the technique described in “Using the 1-cm MicroCuvette Rack” on page 5.



**10.** At the end of the 20-minute period, discard the contents of all the cuvettes into an appropriate waste container.

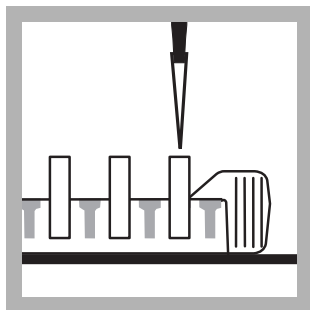


**11.** Wash each cuvette forcefully and thoroughly four times with deionized water. Empty the rinse water into the waste container.

**Note:** Ensure most of the water is drained from the cuvettes by turning the cuvettes upside down and tapping them lightly on a paper towel

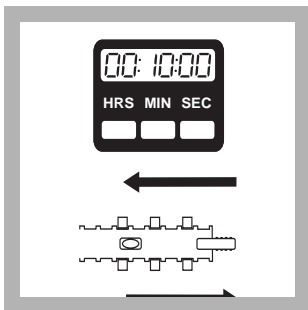
## Color Development

**Note:** Timing is critical; follow instructions carefully.



**12.** With the cuvettes still held snugly in the rack, pipet 0.5 mL of Color Developing Solution into each Antibody Cuvette.

**Note:** Use a new pipette tip for each cuvette.



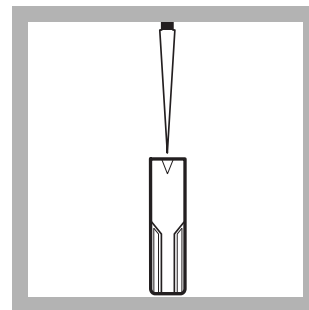
**13.** Key 1000. Press the soft key under **START TIMER**.

A reaction period will begin. Mix following the instructions in *Using the 1-cm MicroCuvette Rack*.



**14.** After 5 minutes, mix the contents of the rack a second time for a period of 30 seconds using the same technique.

**Note:** Solutions will turn blue in some or all of the cuvettes.



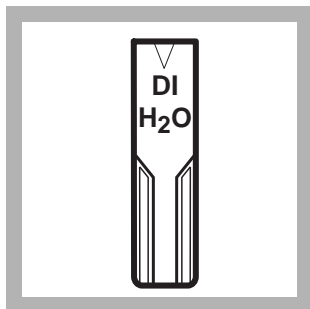
**15.** At the end of the 10-minute reaction period, pipette 0.5 mL of Stop Solution into each cuvette in the same order as the Color Developing Solution was added in step 12.

Slide the rack for 20 seconds using the technique described in *Using the 1-cm MicroCuvette Rack*.

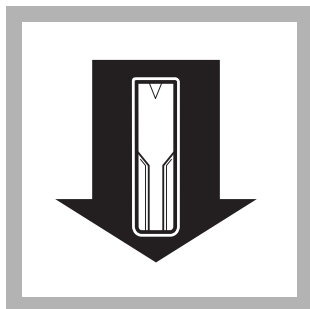
**Note:** Blue solutions will turn yellow with the addition of the Stop Solution.

**Note:** The same pipette tip can be used repeatedly for this step.

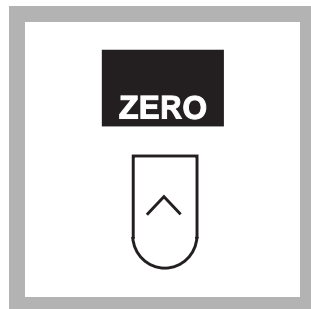
## Measuring the Color



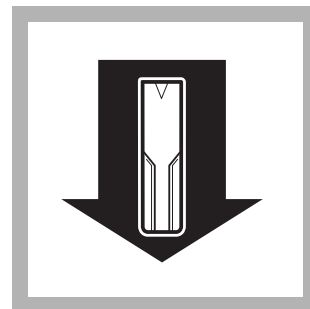
**16.** Label and fill a Zeroing Cuvette with deionized water. Wipe the outside of all the cuvettes with a tissue to remove water, smudges, and fingerprints.



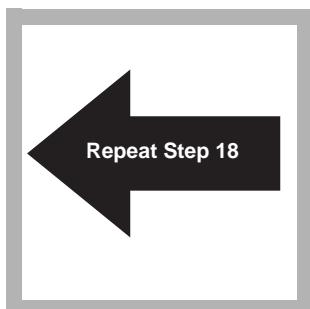
**17.** Place the filled zeroing cuvette into the cell holder with the arrow pointing left. Orient the arrow in the same direction for all cuvettes.



**18.** Press the soft key under **ZERO**.  
The display will show:  
**0.000 Abs**



**19.** Place the first calibrator into the cell holder. Read the results. The display will give an absorbance reading. Record the results for each calibrator and sample.  
**Note:** See the *Instrument Manual* for more information on taking a reading.



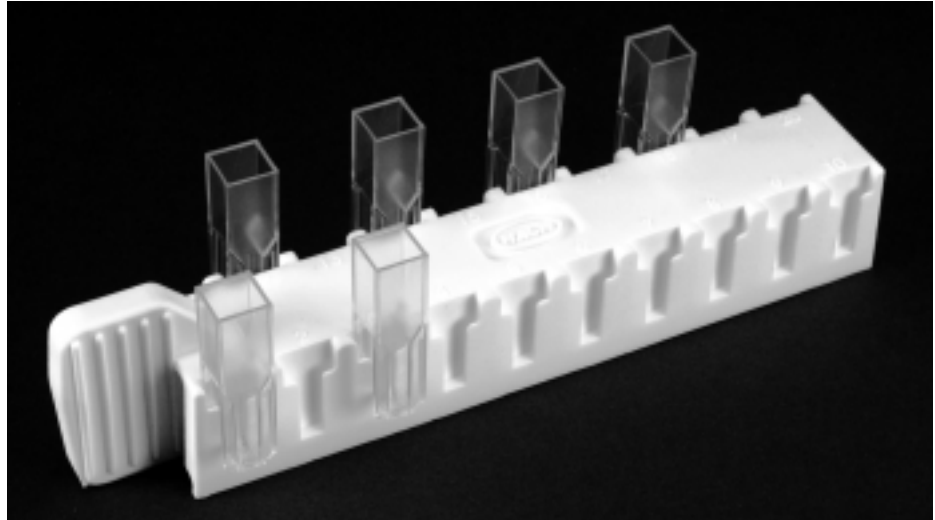
**20.** Repeat *step 19* for all remaining calibrators and samples.

See *Interpreting and Reporting Results* for help with interpretation of results.

## Using the 1-cm MicroCuvette Rack

This rack (see *Figure 1*) has been designed specifically to aid in achieving precise and accurate results when using the immunoassay technique to analyze several samples at the same time.

**Figure 1** The 1-cm MicroCuvette Rack



**Loading the Rack** — The cuvette rack is designed so that it may be inverted with the cuvettes in place. Identify each cuvette with a sample or calibrator number and place all the cuvettes in the rack before beginning the procedure. Fit the cuvettes snugly into the rack, but do not force them or they may be difficult to remove and their contents may spill. The cuvettes should remain in place when the rack is inverted and tapped lightly.

**Mixing** — Set the rack on a hard, flat surface that is at least twice the length of the rack. Hold the rack by one end and vigorously slide it back and forth along its long axis for 30 seconds. The rack should move through a distance equal to its own length in each direction.

## Interpreting and Reporting Results

There is an inverse relationship between the concentration of Atrazine and the reading. In other words, the higher the reading, the lower the concentration of Atrazine.

If the sample reading is...	the sample Atrazine Concentration is...
...less than calibrator reading	...greater than the calibrator concentration
...greater than calibrator reading	...less than the calibrator concentration

### Example

**Readings:**

0.5 ppm Atrazine Calibrator: **0.475 ABS**

2.0 ppm Atrazine Calibrator: **0.245 ABS**

Sample #1: **0.140 ABS**

Sample #2: **0.300 ABS**

Sample #3: **0.550 ABS**

## Interpretation

**Sample #1** — Sample reading is less than the readings for both calibrators. Therefore the sample concentration of Atrazine is greater than both 0.5 ppb and 2.0 ppb Atrazine.

**Sample #2** — Sample reading is between the readings for the 0.5 ppb and 2.0 ppb Atrazine calibrators. Therefore the sample concentration of Atrazine is between 0.5 ppb and 2.0 ppb.

**Sample #3** — Sample reading is greater than the readings for both calibrators. Therefore the sample concentration of Atrazine is less than both 2.0 ppb and 0.5 ppb.

## Storing and Handling Reagents

- Wear protective gloves and eyewear.
- When storing reagent sets for extended periods of time, keep them out of direct sunlight. Store reagents at a temperature of 4 °C when not in use.
- Keep the foil pouch containing the Atrazine Antibody Cuvettes sealed when not in use.
- If Stop Solution comes in contact with eyes, wash thoroughly for 15 minutes with cold water and seek immediate medical help.

## Sensitivity

The Atrazine immunoassay cannot differentiate between the various triazines and metabolites, but it detects their presence in differing degrees.

**Table 1 Required Concentrations for Selected Chemicals**

Compound	Concentration to give a positive result at 0.5 ppb Atrazine	Concentration to give a positive result at 2.0 ppb Atrazine
Acetochlor	74 ppm	398 ppm
Butachlor	84 ppb	550 ppb
2 Chloro-2'6'-Diethylacetaniline	8 ppm	60 ppm
2,6-Diethylaniline	61 ppm	313 ppm
Propachlor	60 ppb	295 ppb

The following compounds are not detectable at 10,000 ppb.

Atrazine	Carbofuran	Carbendazim
Aldicarb	2,4-D	
Diazoton	Chlorpyrifos	

**Sample Collection and Storage**

Collect samples in a clean glass bottle. Do not pre-rinse the bottle with the sample. If the sample cannot be analyzed immediately, store the sample at 4 °C. Samples may be kept for as long as 14 days. Warm the samples to room temperature before analysis.

**Summary of Method**

Hach immunoassay tests use antigen/antibody reactions to test for specific organic compounds in water and soil. Atrazine-specific antibodies, attached to the walls of plastic cuvettes, selectively bind and remove Atrazine from complex sample matrices. A prepared sample and a reagent containing enzyme-conjugate molecules (analyte molecules attached to molecules of an enzyme) are added to the Antibody Cuvettes. During incubation, enzyme-conjugate molecules and Atrazine compete for binding sites on the antibodies. Samples with higher levels of analyte will have more antibody sites occupied by Atrazine and fewer antibody sites occupied by the enzyme-conjugate molecules.

After incubation, the sample and unbound enzyme conjugate are washed from the cuvette and a color-development reagent is added. The enzyme in the conjugate catalyzes the development of color. Therefore, there is an inverse relationship between color intensity and the amount of Atrazine in the sample. The resulting color is then compared with a calibrator to determine whether the Atrazine concentration in the sample is greater or less than the threshold levels.

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**Required Reagents**

Description	Unit	Cat. No.
Reagent Set, Atrazine* .....	20 cuvettes.....	27627-00

**Required Apparatus**

Caps, flip spout.....	2/pkg.....	25818-02
Cell Adapter, 1-cm MicroCell.....	each.....	48588-00
Marker, laboratory.....	each.....	20920-00
Rack, for 1-cm Micro Cuvettes .....	each.....	48799-00
Wipes, disposable.....	box.....	20970-00
TenSette®, Pipet, 0.1–1.0 mL.....	each.....	19700-01
Tips, for pipettor 19700-01 .....	1000/pkg.....	21856-28

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\* Immunoassay components are manufactured for Hach Company by Beacon Analytical Systems, Inc.



**FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:**

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

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**HACH COMPANY**  
WORLD HEADQUARTERS  
Telephone: (970) 669-3050  
FAX: (970) 669-2932