

STRATEGIC DIAGNOSTICS INC.

RaPID Assay[®] Atrazine Test Kit

A00002/A00071

Intended Use

The RaPID Assay[®] Atrazine Test Kit can be used as a quantitative, semi-quantitative or qualitative enzyme immunoassay (EIA) for the analysis of atrazine in water (groundwater, surface water, well water). For applications in other matrices please contact our Technical Service department. The RaPID Assay[®] Atrazine Test Kit allows reliable and rapid screening for atrazine and related compounds, with quantitation, between 0.1 ppb and 5.0 ppb. The minimum detection level of the kit is 0.046 ppb (as atrazine.)

Test Principles

The Atrazine RaPID Assay[®] kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of atrazine and related compounds. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to atrazine attached. Both the atrazine (which may be in the sample) and the enzyme labeled atrazine (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 atrazine and labeled atrazine 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 atrazine is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5' – tetramethylbenzidine). The enzyme labeled atrazine analog bound to the atrazine 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 atrazine (conjugate) was in competition with the unlabeled atrazine (sample) for the antibody sites, the color developed is inversely proportional to the concentration of atrazine in the sample.

NOTE: Color development is inversely proportional to the atrazine concentration.

Darker color = lower concentration

Lighter color = higher concentration

The determination of the atrazine level in an unknown sample is interpreted relative to the standard curve generated from kit standards after reading with a spectrophotometer.

Performance Characteristics

The Atrazine RaPID Assay[®] will detect atrazine and related compounds to different degrees. Refer to the table below for data on several of these compounds. The Atrazine 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 Atrazine RaPID Assay[®] immunoassay test does not differentiate between atrazine and other related compounds. The table below shows compounds at the method detection limit (MDL) which is the lowest concentration of the compound that can be picked up in the assay. The limit of quantitation (LOQ) is an approximate concentration required to yield a positive result at the lowest standard, this is the lowest concentration of the compound that can be quantified in the assay. The IC50 is the concentration required to inhibit one half of the color produced by the negative control. It is also used to calculate cross-reactivity values to similar compounds.

Compound	MDL (ppb)	LOQ (ppb)	IC50 (ppb)
Atrazine	0.046	0.1	0.72
Propazine	0.033	0.1	0.74
Ametryn	0.053	0.05	0.39
Prometryn	0.054	0.09	0.64
Prometon	0.056	0.31	2.22

Desethyl Atrazine	0.062	0.45	3.21
Terbutryn	0.090	0.76	5.50
Terbutylazine	0.310	2.15	15.5
Simazine	0.340	0.68	4.90
Desisopropyl Atrazine	0.800	30.1	217
Cyanazine	1.0	>10000	>10000
6-Hydroxy Atrazine	1.1	20.6	148

*The following compounds demonstrated no reactivity in the Atrazine RaPID Assay[®] at concentrations up to 1000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, alachlor, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metolachlor, metribuzin, pentachlorophenol, picloram, propachlor, terbufos, thiabendazole, and thiophanate-methyl.

The presence of the following substances up to 250 ppm were found to have no significant effect on Atrazine Rapid Assay[®] results: copper, nickel, sulfate, magnesium, calcium, nitrate, and thiosulfate. Humic acid, iron, sulfide, and sulfite were found to have no significant effect up to 100 ppm. In addition, sodium chloride concentrations up to 0.65 M showed no effect on results.

Precautions

- Training is strongly recommended prior to using the RaPID Assay[®] test system. Contact Strategic Diagnostics for additional information.
- Treat atrazine, solutions that contain atrazine, and potentially contaminated samples as hazardous materials.
- Use gloves, proper protective clothing, and methods to contain and handle hazardous material where appropriate.
- Reagents must be added in a consistent manner to the entire rack. A consistent technique is the key to optimal performance. Be sure to treat each tube in an identical manner.
- Water samples should be at a neutral pH prior to analysis. Samples containing gross particulate should be filtered (e.g. 0.2 um Anotop[™] 25 Plus, Whatman, Inc.) to remove particles.
- Store all test kit components at 2°C to 8°C (36°F to 46°F). Storage at ambient temperature (18°C to 27°C or 64°F to 81°F) on the day of use is acceptable. *Test*

tubes require no special storage and may be stored separately to conserve refrigerator space.

- Allow all reagents to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before beginning the test. This typically requires at least 1 hour to warm from recommended storage conditions.
- Do not freeze test kit components or expose them to temperatures above 100°F (39°C).
- Do not use test kit components after the expiration date.
- Do not use reagents or test tubes from one test kit with reagents or test tubes from a different test kit.
- Do not mix reagents from kits of different lot numbers.
- Use approved methodologies to confirm any positive results.
- Do not under any circumstances attempt to disassemble the base of the magnetic rack. Magnets will be violently attracted to each other.
- Adequate sample number and distribution are the responsibility of the analyst.
- The photometer provided in the accessory kit requires electricity and comes with a 110V adapter. Adapters for 220V are available.
- Do not expose color solution to direct sunlight.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
- Tightly recap the standard vials when not in use to prevent evaporative loss.

Materials Provided

- Antibody Coupled Paramagnetic Particles in buffered saline containing preservative and stabilizers.
30 test kit: one 20 mL vial
100 test kit: one 65 mL vial
- Enzyme Conjugate.

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

- Standards

Three concentrations (0.1, 1.0, 5.0 ppb) of atrazine standards in buffered saline containing preservative and stabilizers are supplied. Each vial contains 4 mL.

- Control

A concentration (approximately 3 ppb) of atrazine in buffered saline containing preservative and stabilizers. A 4 mL volume is supplied in one vial.

- Diluent/Zero Standard

Buffered saline containing preservative and stabilizers without any detectable atrazine.

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

- Color Solution containing 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

- Stop Solution containing a solution of 2 M sulfuric acid.

30 test kit: one 20 mL vial
 100 test kit: one 60 mL vial

- Washing Solution containing preserved deionized water.

30 test kit: one 70 mL vial
 100 test kit: one 250 mL vial

- Polystyrene test tubes

30 test kit: one 36 tube box
 100 test kit: three 36 tube boxes

- User's Guide

Materials Required and Ordered Separately

See "Ordering Information" for the appropriate catalogue numbers.

Rapid Assay® Accessory Kit

Accessory equipment may be rented or purchased from Strategic Diagnostics. See "Ordering Information" for the appropriate catalogue numbers.

The accessory kit contains the following items:

- Adjustable Volume Pipet
- Eppendorf™ Repeater® Pipettor
- Electronic timer
- Portable balance capable of weighing 10 g (for soil samples)
- Vortex mixer
- Magnetic separation rack
- RPA-I RaPID Analyzer (or equivalent spectrophotometer capable of reading 450 nm in a 1 mL sample size).

Other Items

- 12.5 mL Combitips® for the Repeater pipettor - for 0.25 mL to 1.25 mL dispensing volumes (5)
- Pipet tips for adjustable volume pipet (100-1000 uL)

NOTE: Order replacement Combitips® and pipet tips separately. See the "Ordering Information" section.

Materials Required but Not Provided

- Protective clothing (e.g., latex gloves)
- Absorbent paper for blotting test tubes
- Liquid and solid waste containers
- Marking pen
- Instructional video (optional)

Suggestions for Pipettor Use

- Practice using both pipettes (adjustable volume and Repeater pipettor) with water and extra tips before you analyze your samples.
- Use a new tip each time you use the Repeater pipettor to pipette a different reagent to avoid reagent cross-contamination. Tips can be rinsed thoroughly, dried completely and reused. By using the same tip to

dispense the same reagent each time you can avoid cross contamination.

NOTE: Repeater tips should be changed periodically (after ~10 uses) since precision deteriorates with use.

- Draw the desired reagent volume into the Repeater pipettor and dispense one portion of the reagent back into the container to properly engage the ratchet mechanism. If you do not do this, the first volume delivered may be inaccurate.
- To add reagents using the Repeater pipettor, pipette down the side of the test tube just below the rim.
- When adding samples and standard using the positive displacement pipettor, always pipette into the bottom of the tube without touching the sides or bottom of the tube.
- Use a new adjustable volume pipet tip each time you pipette a new unknown.

Assay Procedure

Prior to performing your first Rapid Assay®, please take time to read the package inserts in their entirety and review the videotape if available. On site training is strongly recommended for new users of this test system. Please contact your account manager for further information.

Collect/Store the Sample

The following steps explain how to properly collect and store your samples.

1. Samples should be collected in appropriately sized and labeled containers.
2. If testing soil samples, follow the SDI Sample Extraction Kit User's Guide or the appropriate tech bulletin, to properly collect and store your sample.
3. Water samples should be collected in glass VOC vials with minimal head space.
4. Samples should be tested as soon as possible after collection. If this is not possible, storage at 4°C (39°F) is recommended to minimize evaporative losses.

Set Up

1. Remove kits from refrigerator. All reagents must be allowed to come to room temperature prior to analysis. Remove reagents from packaging and place at room temperature at least 1 hour prior to testing.
2. Turn on the RPA-1 or other spectrophotometer. The RPA-1 should be warmed up for at least 30 minutes prior to the run.
3. Label five 12.5 mL Combitips "Conjugate", "Particles", "Wash", "Color" and "Stop". In addition, add the name of the compound you are testing for to each Combitip.
4. Remove nine clean blank test tubes for standards and control and one test tube for each sample (if testing in singlicate). Label the test tubes according to contents as follows.

<u>Tube #</u>	<u>Contents</u>
1	Negative control (replicate 1)
2	Negative control (replicate 2)
3	Standard 1 (replicate 1)
4	Standard 1 (replicate 2)
5	Standard 2 (replicate 1)
6	Standard 2 (replicate 2)
7	Standard 3 (replicate 1)
8	Standard 3 (replicate 2)
9	Control
10	Sample 1
11	Etc.

***Label at top of tubes to avoid interference with reading of tubes in photometer**

Sample Extraction and Dilution

Water samples being tested at standard kit detection levels do not require extraction. Filtration may be necessary to remove gross particulate from the sample. If testing at levels higher than standard kit levels is desired, contact SDI for special instructions. Please follow the instructions from the SDI Sample Extraction Kit to prepare and dilute the soil extract prior to running the assay.

Perform the Test

1. Separate the upper rack from the magnetic base. Place labeled test tubes into the rack.

2. Add **200 uL** of standards, control or samples to the appropriate tubes using the adjustable volume pipet with the dial set on **0200**. The standards and control must be run with each batch of samples.

NOTE: Sample should be added to the bottom of the tube by inserting the pipet tip into the tube without touching the sides or the bottom of the tube. Take care not to contact sample with pipette tip once dispensed into bottom of the tube.

3. Using the Repeater Pipettor with the “Conjugate” tip attached and the dial set on **“1”**, add **250 uL** of Enzyme conjugate down the **inside wall** of each tube. (Aim the pipet tip $\frac{1}{4}$ ” to $\frac{1}{2}$ ” below the tube rim or tube wall; deliver liquid gently to avoid splashback.)
4. Thoroughly mix the magnetic particles by swirling (avoid vigorous shaking) and attach the “Particles” tip to the Repeater Pipettor. With the dial set on **“2”** add **500 uL** of magnetic particles to each tube, aiming down the side of the tube as described above. Vortex, mixing each tube 1 to 2 seconds at low speed to minimize foaming. Pipetting of magnetic particles should be kept to 2 minutes or less.
5. Incubate 15 minutes at room temperature.
6. After the incubation, combine the upper rack with the magnetic base and press all tubes into the base; allow 2 minutes for the particles to separate.
7. With the upper rack and magnetic base combined, use a smooth motion to invert the combined rack assembly over a sink and pour out the tube contents.

NOTE: If the rack assembly inadvertently comes apart when lifting to pour out tube contents, recombine and wait an additional 2 minutes to allow particles to separate.

8. **Keep the rack inverted** and gently blot the test tube rims on several layers of paper towels. It is important to remove as much liquid as possible but **do not bang** the rack or you may dislodge the magnetic particles and affect the results.
9. Set the Repeater Pipettor dial to **“4”** and put on the tip labelled “Wash”. Add **1 mL** of Washing Solution down the inside wall of each tube by using the technique described earlier. Wait 2 minutes and pour

out the tube contents as described previously. **Repeat this step one more time.**

NOTE: The number of washes and wash volume are important in ensuring accurate results.

10. Remove the upper rack (with its tubes) from the magnetic base. With the “Color” tip attached to the Repeater Pipet and the dial set to **“2”** add **500 uL** of Color Reagent down the inside wall of each tube as described previously. Vortex 1 to 2 seconds (at low speed).
11. Incubate 20 minutes at room temperature. During this period, add approximately 1 mL of Washing solution to a clean tube for use as an instrument blank for “Results Interpretation”.
12. After the incubation, position the Repeater pipettor at Setting **“2”** and use the “Stop” tip to add **500 uL** of Stop solution to all test tubes.
13. Proceed with results interpretation.

WARNING: Stop solution contains 2M sulfuric acid. Handle carefully.

Results Interpretation

1. After addition of Stop Solution to the test tubes, results should be read within 15 minutes.
2. Wipe the outside of all antibody coated tubes prior to photometric analysis to remove fingerprints and smudges.

Photometric Interpretation Using the RPA-I

1. The RPA-I photometer (provided in the Rapid Assay[®] Accessory kit) can be used to calculate and store calibration curves. It is preprogrammed with various RaPID Assay[®] protocols. To obtain results from the Atrazine 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: 0.10 ppb
 #3: 1.00 ppb
 #4: 5.00 ppb
 Range : 0.05 – 5.00
 Correlation : 0.990
 Rep. %CV : 10%

NOTE: Tube order is important. The RPA-I expects to see the standards in ascending order, in duplicate, starting with the negative control.

Following evaluation of all standards, the instrument will display:

PRINTING DATA,	Data will print
PRINTING CURVE	Curve will print only if programmed to print (See RPA1 User's Manual).
CTRL #1 REP #1, INSERT TUBE, EVALUATING TUBE, REMOVE TUBE (Beep)	Insert Control Tube Remove Tube
EDIT CALIBRATORS YES/NO	Press NO (if editing is necessary press YES and refer to the RPA1 User's Manual).
SPL #1 REP#1 INSERT TUBE EVALUATING TUBE REMOVE TUBE (Beep)	Insert first sample tube Remove tube

NOTE: Prior to analysis the RPA-I User's Manual should be thoroughly reviewed for more detailed operation instructions.

2. Follow the instrument prompts to read the absorbance of all tubes:

<u>Instrument Display</u>	<u>Operator Response</u>
SELECT COMMAND RUN PROTOCOL	Press RUN Scroll using the YES [] or NO [] keys until the desired protocol appears. Then press ENTER
SPL. REPLICATES (1-5)	Press 1 (for analysis of samples in singlicate.) Press ENTER
BLANK TUBE, INSERT TUBE, EVALUATING TUBE, REMOVE TUBE (Beep)	Insert blank tube containing 1mL wash solution. Remove tube
CAL #1, REP. #1, INSERT TUBE, EVALUATING TUBE, REMOVE TUBE (Beep)	Insert Tube #1 Remove tube

Continue to follow prompts. After all samples have been read, press STOP.

Expected Results:

- **%CV (coefficient of variation) between standard duplicates of 10% or less.**
- **Absorbance reading for the 0 ppb standard should be between 0.8 and 2.0 for all assays.**
- **Correlation (r) of 0.990 or greater for all assays.**
- **Kit control within range specified on vial.**
- **Absorbance of negative control and standards should be as follows:**

Negative Control > Std. 1 > Std. 2 > Std. 3.

3. Concentrations will be indicated for all samples on the RPA-I printout.

Follow prompts to read tubes.

- a) Samples with an “nd” and no concentration listed have an absorbance greater than the

negative control; therefore, no concentration can be computed for these samples. Results must be reported as <0.1 (Standard 1).

- b) Samples with an “nd” next to a listed concentration have an estimated concentration below the minimum detection level of the test kit. Results must be reported as <0.1 (Standard 1).

NOTE: Any samples with concentrations determined to be lower than Standard 1 (the limit of quantitation) must be reported as <0.1 ppb. Quantitation is not possible below this standard as this is outside the linear range of the assay.

- c) Similarly, samples with a “hi” next to a listed concentration have an estimated concentration higher than Standard 3 and must be reported as >5.0 ppb.

NOTE: In order to determine the concentration of samples with concentrations greater than Standard 3, they must be subjected to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of atrazine diluent. This additional dilution must then be taken into account when calculating the concentration.

- d) The concentration, as indicated on the printout, is multiplied by the appropriate dilution factor (if applicable) introduced in the procedure. For example, if the sample was diluted a total of 10x, the concentrations listed on the printout should be multiplied by 10 to determine the appropriate sample concentration.

Photometric Interpretation Using Other Photometers

Other photometers may also be used to interpret results obtained from the RPA-I photometer. It is important that the photometer be able to read absorbance at 450nm and that the instrument can read at a 1 mL fill volume.

Absorbances obtained from other spectrophotometers (reading at 450 nm) may be used to manually calculate sample concentrations as outlined below.

1. Calculate the mean absorbance for each of the three standards and the negative control.
2. Determine the standard deviation and %CV (coefficient of variation) of each standard and ensure %CV is less than 10% for each.
3. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the negative control and multiplying the results by 100.
4. Construct a standard curve by plotting the %B/Bo for each standard on the vertical logit (y) axis versus the corresponding analyte concentration on the horizontal logarithmic (x) axis on the graph paper provided in the test kit. **Graph papers are specific for each method. Use only the graph paper supplied with each kit.**
5. Draw the best straight line through all points. Using the %B/Bo of the sample, the concentration can be interpolated from the standard curve.
6. Multiply results by the appropriate dilution factor (if applicable) introduced in the procedure. For example, if the sample was diluted 10-fold to increase the detection levels of the kit then the results must be multiplied by 10. This dilution also changes the range of the assay (standards) by the same factor. So, if the 10 fold dilution were made, the range of the kit would now be 1.0 ppb to 50 ppb.

Limitations of the Procedure

The Rapid Assay® Atrazine Test Kit is a screening test **only**. Sampling error may significantly affect testing reliability. Adequate sample number and distribution are the responsibility of the analyst.

Ordering Information

Description	Catalogue Number
Rapid Assay [®] Atrazine 30 Tube Kit	A00002
Rapid Assay [®] Atrazine 100 Tube Kit	A00071
Rapid Assay [®] Accessory Kit**	6050100
Adjustable Volume Pipet Tips (100-1000 uL)	A00013
12.5 mL Combitip for Repeating Pipette (1 each)	A00009
Atrazine Diluent	A00051
Rapid Assay [®] Accessory Kit Rental	6997010
** To obtain part numbers and pricing for individual items in the Accessory Kit contact SDI at the number below.	

Ordering/Technical Assistance

Should you have any questions regarding this procedure prior to analysis contact Technical Service to avoid costly mistakes.

To Place an Order or Receive Technical Assistance, please call Strategic Diagnostics Inc. at:

Call toll-free **800-544-8881**

Or 302-456-6789 Phone

302-456-6782 Fax

Web site: www.sdix.com

E-mail: techservice@sdix.com

General Limited Warranty

SDI's products are manufactured under strict quality control guidelines and are warranted to be free from defects in materials and workmanship. New instruments and related non-expendable items are warranted for one year from date of shipment against defective materials or workmanship under normal use and service.

Warranty obligation is limited to repair or replacement of the defective product or to refund of the purchase price, at the discretion of SDI. Other warranties, express or implied, are disclaimed. SDI's liability under any warranty claim shall not exceed the refund of the purchase price paid by the customer. Under no circumstances shall SDI be liable for special, indirect or consequential damages.

Safety

To receive an MSDS for this product, visit our web site at www.sdix.com.

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Operation of the Repeater Pipet

To Set or Adjust Volume

To determine the pipetting volume, the dial setting (1-5) is multiplied by the minimum pipetting volume of the tip (indicated on the side of the Combitip, e.g. 1 \approx 100 uL.)

To Assemble Pipet Tip

Slide filling lever down until it stops. Then raise the locking clamp and insert the tip until it clicks into position. Be sure the tip plunger is fully inserted into the barrel before lowering the locking clamp to affix the tip in place.

To Fill Tip

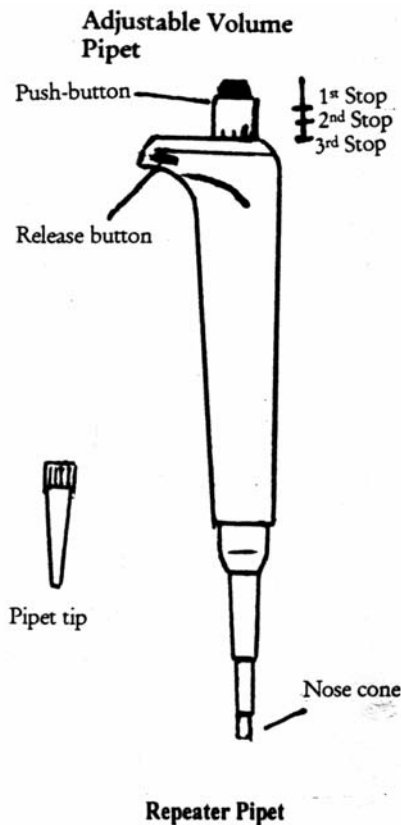
With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly. Combitip will fill with liquid.

To Dispense Sample

Check the volume selection dial to ensure pipetting volume. Place tip inside test tube so that tip touches the inner wall of tube. Completely depress the pipetting lever to deliver sample. NOTE: Dispense one portion of reagent back into the container to engage the ratchet mechanism and ensure accuracy.

To Eject Tip

Empty tip of any remaining solution into appropriate container by pushing filling lever down. Raise locking clamp upward, and remove the Combitip.



Operation of the Adjustable Volume Pipet

To Set or Adjust Volume

Press release button on side of pipette and turn the push-button to adjust volume up or down. Volume setting is displayed on top of pipet. See kit instructions for appropriate setting. Pipet will accurately dispense volumes between 100 and 1000 uL.

To Assemble Pipet Tip

Gently push nose cone of pipet firmly into a pipet tip contained in the pipet tip rack.

To Withdraw Sample

Keep pipet almost vertical. With tip mounted in position on pipet, press push-button to 1st stop and hold it. Place tip at bottom of liquid sample and slowly release push-button to withdraw measured sample. Ensure that no air bubbles exist in the pipette tip. If bubbles exist, dispense sample and re-withdraw. Slide tip out along the inside of the vessel.

To Dispense Sample

Wipe any liquid from outside of tip taking care not to touch orifice. Place tip into tube, almost to the bottom, and slowly press push-button to 2nd stop. Hold push-button at 2nd stop when removing tip from tube.

To Eject Tip

Press push-button to 3rd stop. Tip is ejected.