STRATEGIC DIAGNOSTICS INC.

Ensys™ PAH Soil Test Kit, EPA Method 4035
7061301

Intended Use

The Ensys PAH Soil Test Kit is a qualitative or semi-quantitative enzyme immunoassay (EIA) for the analysis of polyaromatic hydrocarbons (PAH) at user specified detection levels in soil. The method correctly identifies 95% of samples that are PAH-free and those containing 1 ppm total PAH (as phenanthrene).

Background

Contamination of soil with polyaromatic hydrocarbons (PAH) is a serious problem at manufactured gas plant sites, coking operations, wood preserving operations that have used creosote as a wood preservative, and petrochemical plant waste disposal sites. The federal and state regulatory agencies are mandating the clean-up of many of these sites due to the carcinogenic nature of some PAHs.

Polyaromatic hydrocarbons, or polynuclear aromatic hydrocarbons (PAHs), are fused ring aromatic compounds classified by the number of carbon rings. The EPA lists sixteen of these PAHs as hazardous compounds. They are further divided into carcinogenic and non-carcinogenic PAHs. The two and three ring PAHs are non-carcinogenic, while several of the four, five and six ring PAHs are carcinogenic. The four ring PAHs, chrysene and benzo[a]anthracene, the five ring PAHs, benzo[a]pyrene, benzo[b]fluoranthene, benzo [k] fluoranthene and dibenzo [a,h] anthracene, and the six ring PAH, indeno [1,2,3-cd] pyrene, are carcinogenic PAHs. Benzo [a] pyrene is the most potent carcinogen among the PAHs. Regulatory concern is generally focused on benzo [a] pyrene, total carcinogenic PAHs and total PAHs.

Test Principles

The Ensys PAH Soil Test Kit is based on the use of antibodies that bind either PAH or PAH-Enzyme Conjugate. These antibodies are immobilized on the walls of the test tubes. When PAH is present in the sample, it competes with the PAH-Enzyme Conjugate for a limited number of PAH binding sites on the immobilized antibodies.

- A sample containing PAH is added to a test tube containing PAH-Enzyme Conjugate. The PAH-Enzyme Conjugate competes with the PAH for the antibody binding sites.
- After incubation, the unbound molecules are washed away.
- A clear solution of chromogenic Substrate is then added to the test tube. In the presence of bound PAH-Enzyme Conjugate, the clear Substrate is converted to a blue color. One enzyme molecule can convert many Substrate molecules.

Since every test tube has the same number of antibody binding sites and receives the same number of PAH-Enzyme Conjugate molecules, a sample that contains a low concentration of PAH allows the antibody to bind many PAH-Enzyme Conjugate molecules. Therefore, a low concentration of PAH produces a dark blue solution. Conversely, a high concentration of PAH allows fewer PAH-Enzyme Conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

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

Darker color = lower concentration
Lighter color = higher concentration

The determination of the PAH level in an unknown sample is interpreted relative to the kit standard using visual comparison or by reading with a spectrophotometer. The standard is at a fixed concentration; therefore, the kit detection levels are determined by the dilution of the sample being analyzed. Dilution ampules are provided in the test kit based on the detection level(s) specified at the time of ordering.

Performance Characteristics

The Ensys PAH Soil Test Kit can be used to screen soil samples for the presence of both carcinogenic and non-

carcinogenic PAH compounds. Because the PAH test does not measure all of the sixteen listed PAHs, nor does it measure exclusively the carcinogenic PAHs, it should be viewed as an indicator or screening test for PAHs. In order to ensure that a user is likely to obtain good correspondence with confirming laboratory data, it is advisable to review any analytical data that has been obtained prior to doing extensive field screening. For example, abnormally high levels of naphthalene could lead to underestimation of total PAHs. This will help to eliminate user issues before they occur. The table below indicates the chemical cross reactivity of the PAH Soil Test kit:

PAH Compound	Concentration Necessary to Result in Positive Test (ppm)*
Naphthalene	200 ppm
Acenaphthene	8.1 ppm
Acenaphthylene	7.5 ppm
Phenanthrene	1.0 ppm
Anthracene	0.81 ppm
Fluorene	1.5 ppm
Benzo(a)anthracene	1.6 ppm
Chrysene	1.2 ppm
Fluoranthene	1.4 ppm
Pyrene	3.5 ppm
Benzo(b)fluoranthene	4.6 ppm
Benzo(k)fluoranthene	9.4 ppm
Benzo(a)pyrene	8.3 ppm
Dibenzo(a,h)anthracene	>200 ppm
Indeno(1,2,3-cd)pyrene	11 ppm
Benzo(g,h,I)perylene	>200 ppm

*Samples with stated concentration will give positive result greater than 95% of the time when tested at stated concentration level.
">" means that no positive results were observed at the stated concentration and this concentration was the highest tested.
**Kit standard is phenanthrene.

Precautions

- Treat PAH, solutions that contain PAH, and potentially contaminated soil samples as hazardous materials.
- Use gloves, proper protective clothing, and methods to contain and handle hazardous material where appropriate.
- Store all test kit components at ambient temperature (18°C to 27°C or 64°F to 81°F). Do not freeze test kit components or expose them to temperatures above 100°F (39°C).
- This test kit should be operated between 40°F (4°C) and 90°F (32°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.
- Soils obtained from areas adjacent to standing water, surface soils collected during or immediately after rain or snow, or any soils with relatively high amounts of water ($\geq$ 30% by weight) should be dried before testing. Contact technical service for recommended methods.
- Distribution of PAH in soils may be highly variable. Adequate sample number and distribution are the responsibility of the analyst.
- Portable spectrophotometer battery must be fully charged prior to use. It will not run directly off of AC current.
- Do not expose substrate 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 PAH calibrator vials to prevent evaporative loss.

Materials Provided

- 48 Antibody coated test tubes (12 x75) in foil pouches
- 2 ampules of PAH Standard (phenanthrene in methanol)
- 48 PAH-Enzyme Conjugate vials w/ gray stoppers

- 15 mL bottle of Substrate A
- 15 mL bottle of Substrate B
- 15 mL bottle of Stop Solution
- 480 mL bottles of Wash Solution (2)
- 24 Pink (50-250 µL) Gilson Microman® positive displacement pipette tips
- User's Guide
- Bulb pipettes (3)
- Amber vials with screw caps (3)
- 60 mL bottle of Buffer
- 12 Small ampule crackers
- 5.0 mL Combitips for Repeater pipettor (3)
- 12.5 mL Combitip for Repeater pipettor

Materials Required and Ordered Separately

See "Ordering Information" for the appropriate catalogue numbers.

SDI Sample Extraction Kit

Use this kit for the extraction of PAH from soil samples. This kit contains enough devices to process 12 samples:

- 12 Extraction jars with screw caps (each bottle contains 3 stainless steel mixing beads)
- 12 Filter modules (tops and bottoms)
- 12 Ampule crackers
- 12 Wooden spatulas
- 12 Weigh Canoes
- 12 Disposable Transfer Pipettes
- 12 Ampules containing 20 mL each of 100% Methanol
- Dilution series with ampules containing required volume of methanol to achieve user defined detection levels

Ensys/Envirogard Field Soil Lab (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:

- Gilson M-25 Microman Positive Displacement Pipettor
- Eppendorf™ Repeater® Pipettor
- Electronic timer
- Polystyrene test tubes, 12 x 75 mm (for blanking spectrophotometer)
- Portable balance capable of weighing 10 g
- Wash bottle
- 5.0 mL Combitips® for the Repeater pipettor -for 0.1 mL to 0.5 mL dispensing volumes (3)
- 12.5 mL Combitips® for the Repeater pipettor -for 0.25 mL to 1.250 mL dispensing volumes (6)
- 50.0 mL Combitip® for the Repeater pipettor (with adapter)-for 1.0 mL to 5.0 mL dispensing volumes (1)
- Foam workstation
- Differential photometer - allows you to measure results in the form of optical density values. These values can be used for objective record keeping and quality assurance.

NOTE: Order replacement Combitips® and positive displacement 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

Suggestions for Pipettor Use

- Practice using both pipettors (positive displacement 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 and

reused. By using the same tip to dispense the same reagent each time you can avoid cross contamination.

- 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 below the liquid level. Pipet liquid up and down in tip to ensure complete volume transfer.
- The carryover volume of the positive displacement tips is minimal, but may affect results if you are going from a high to low PAH concentration. Use a new pipettor tip each time you pipette a new unknown.

Assay Procedure

Collect/Store the Sample

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

1. Collect soil in appropriately sized and labeled containers.
2. Take care to remove excess twigs, organic matter, and rocks or pebbles from the soil sample to be tested.
3. Soils obtained from areas adjacent to standing water, surface soils collected during or immediately after rain or snow, or any soils with relatively high amounts of water ($\geq$ 30% by weight) should be dried before testing. Contact Technical Services for recommended methods.
4. Store soil samples at 4°C (39°F).

Workstation Set Up

1. Open one of the ampules labeled "PAH Standard" by slipping the ampule cracker over the top and breaking the tip at the scored neck. Transfer the solution in the ampule to one of the empty amber vials using a bulb pipet and cap the vial. The vial should then be labeled with the current date.

NOTE: The standard is good for two weeks after being transferred from the ampule. After two weeks, a new standard ampules should be opened.

2. Label the three 5.0 mL Combitips found in your Penta Soil test kit "A", "B" and "Stop". Label the larger 12.5 mL Combitip "Buffer".
3. Set up the workstation as indicated on Page 7 of this User's Guide.
4. Label the conjugate tubes and antibody coated tubes as follows (This is an example of how tubes might be labelled for 1 and 10 ppm detection levels.) Do not attempt to run more than 12 tubes per assay, two of which must be standards.:

Tube Label	Tube Contents
Std1	Standard (replicate 1)
Std2	Standard (replicate 2)
#1 – 1 ppm	Sample 1 (1 ppm detection)
#1 - 10 ppm	Sample 1 (10 ppm detection)
#2 – 1 ppm	Sample 2 (1 ppm detection)
#2 – 10 ppm	Sample 2 (10 ppm detection)
#3 - 1 ppm	Sample 3 (1 ppm detection)
Etc.	

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

Extract the Soil/Dilute the Sample

1. Follow the instructions from the SDI Sample Extraction Kit to prepare the soil extract before the assay. **20 mL** of **100 % Methanol** will be used to extract PAH residue from a **10 g** soil sample.
2. Uncap conjugate tubes in Row 2. Position the Repeater pipettor at Setting **4** and use the 12.5 mL "Buffer" tip to add **1 mL** of buffer to all conjugate tubes.
3. Open a series of dilution ampules in Row 1 for each sample to be tested by slipping an ampule cracker over the top and breaking at the scored neck.

NOTE: If your kit includes intermediate dilution ampules to reach your detection level they should be opened for each sample as well.

4. Attach a clean pink pipette tip to the positive displacement pipet and adjust the dial to **"075"** to pipet **75 µL**. Use the pipettor to withdraw 100 uL of

filtered sample extract from the filter unit. Transfer to the dilution ampule with the lowest ppm level. Gently shake ampule from side to side for 5 seconds to mix thoroughly.

5. Withdraw **75 uL** of diluted sample from the first dilution ampule using the positive displacement pipet and transfer to the next highest dilution ampule provided in your kit. Gently shake the ampule from side to side for 5 seconds to mix thoroughly. Continue this procedure for all ampules provided in the dilution series, transferring from the lowest to highest ppm value.

EVERY AMPULE PROVIDED IN THE DILUTION SERIES MUST BE USED IN ORDER TO ACHIEVE YOUR TEST LEVELS!

6. After all dilutions have been made, use the same pipet tip used for dilution to transfer **75 uL** from each dilution ampule to the corresponding conjugate tube in Row 2.

NOTE: Always begin transfers from ampules to conjugate tubes starting with the highest ppm dilution ampule and working to the to the lowest ppm ampule. Wipe the tip of the pipet after dispensing to minimize cross contamination. Do not transfer from dilution ampules which are not at your desired testing levels to conjugate tubes as this uses reagents and reduces the number of samples obtained per kit.

7. Repeat Steps 4-6 for each sample to be tested, using a clean pipette tip for each new sample.

8. Assemble a new pipette tip on the positive displacement pipette and transfer **75 uL** from the standard vial into each of two corresponding conjugate tubes in Row 2.

CAUTION: Replace the cap(s) on the standard vials immediately after use to minimize evaporation.

9. Gently shake all of the conjugate tubes for 5 seconds to mix.

Perform the Test

1. Fit all antibody coated tubes in Row 3 firmly on top of all corresponding conjugate tubes in Row 2. Set a timer for 10 minutes, start the timer and immediately invert all connected tube pairs, working left to right in the workstation. This will transfer buffer to the antibody coated tube. Make sure the plastic antibody coated tube is on the bottom.

2. Again working left to right in the workstation, invert the connected tube pairs three more times, making sure the antibody coated tubes are on the bottom and seated in Row 2 when complete.

3. Disconnect and discard the smaller conjugate tubes. Do not worry about drops of liquid adhering to the lips of the tubes.

4. After the 10 minute incubation, vigorously shake out the test tube contents into a sink or suitable container. Wash the tubes by vigorously filling and emptying a total of four times with the Wash Solution provided in the test kit. After the last wash, tap the tubes upside down on paper towels to remove excess liquid. (Residual foam will not interfere with results.)

5. Position the Repeater pipettor at Setting **2** and use the **5.0 mL** Combitip labeled "A" to add **200 µL** of Substrate A to all test tubes.

6. Set the timer for exactly 2 ½ minutes but do not start it.

7. Assemble the **5.0 mL** "B" tip on the Repeater pipettor at Setting **2** and fill the tip with Substrate B.

8. Start the timer and use the Repeater pipettor to add **200 uL** of Substrate B to all test tubes. Shake all tubes for 5 seconds. Solution will turn blue in some or all of the tubes.

9. After the 2 ½ minute incubation, position the Repeater pipettor at Setting **2** and use a **5.0 mL** Combitip to add **200 µL** of Stop Solution to all test tubes. This will turn the color from blue to yellow.

WARNING: **Stop solution contains sulfuric acid. Handle carefully.**

Results Interpretation

You can either interpret the results visually within 5 minutes after adding the Substrate to each test tube, or you

can perform a more precise analysis with a photometer after you add the Stop Solution.

Visual Interpretation

After you add the Substrate, wait 5 minutes then mix the test tubes by shaking them for a few seconds. Compare the sample test tube to the lighter standard tube against a white background.

- If a sample test tube contains *more* color than the standard test tube, the sample contains PAH at a concentration *lower* than the level being tested.
- If a sample test tube contains *less* color than the standard test tube, the sample may contain PAH at a concentration *greater* than the level being tested.

Photometric Interpretation

NOTE: After you add Stop Solution to the test tubes, results should be read within 30 minutes.

1. Dry the outside of all antibody coated tubes prior to photometric analysis.

2. Place both standard tubes in the differential photometer.

3. Switch the tubes until the photometer reading is negative or zero. Record the reading.

NOTE: The standard is run in duplicate to provide internal test system quality control. With both standards inserted in the photometer, a valid test is indicated when the magnitude of the displayed number (irrespective of the sign + or -) is less than 0.30. If the number obtained is greater than 0.30 the results are outside QC limits and the test should be repeated to ensure valid conclusions.

4. Remove and discard the tube in the right well of the photometer. The tube in the left well is the conservative standard to which your sample tubes will be compared.

5. Place the antibody coated tubes corresponding to each sample into the right well of the photometer one at a time and record the readings.

    - If the photometer reading is negative or zero, PAH is present at a level greater than or equal to the testing level for that sample.
    - If the photometer reading is positive, the concentration of PAH is less than the testing level for that sample.

Limitations of the Procedure

The Ensys PAH Soil Test Kit is a screening test **only**. Soil sampling error may significantly affect testing reliability. The distribution of PAH in soils can be extremely heterogeneous. Adequate sample number and distribution are the responsibility of the analyst.

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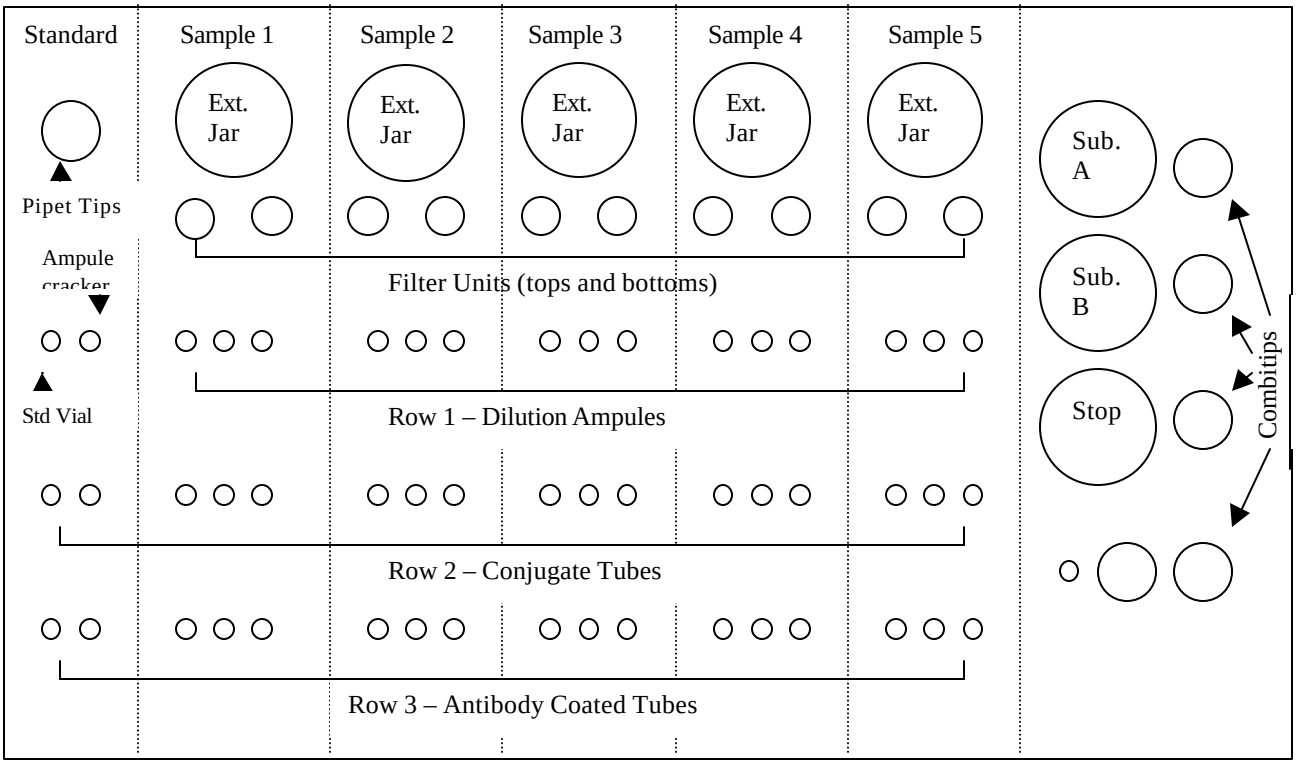
Ensys PAH Soil Workstation Set Up



1. Remove foam workstation from Ensys / Envirogard Field Accessory Kit.
2. Open the SDI Sample Extraction Kit and remove an extraction jar for each sample to be tested. Place in the foam workstation as indicated on the diagram above. The extraction kit also contains bulb pipets which may be placed in the small hole to the left of each extraction jar (not shown).
3. Place a filter unit (top and bottom) from the extraction kit under each extraction jar in the workstation corresponding to each sample.
4. Remove the box of dilution ampules contained in the SDI Sample Extraction kit and place a complete dilution series from the box for each sample to be tested under the filter units corresponding to each sample in the workstation.

   NOTE: A dilution series includes ampules for each level ordered as well as any intermediate levels needed to obtain your desired detection level. EVERY AMPULE IN THE SERIES MUST BE USED IN ORDER TO REACH YOUR DETECTION LEVELS.

5. Place the standard prepared in Step 1 of "Workstation Set Up" in the Ensys PAH Soil Test Kit User's Guide into the hole on the far left of Row 1 in the workstation as indicated on the diagram. Place one of the small ampule crackers provided in the Ensys PAH Soil Test Kit into the hole next to the standard.
6. Into Row 2 place conjugate tubes (which are the gray stoppered vials with white pellets in your test kit) for the desired testing levels for every sample to be tested. (A conjugate tube should not be added for intermediate levels included in the dilution series.) In the two left holes on Row 2 place two conjugate tubes for your standard.
7. Antibody coated tubes, which are in the foil pouch in your test kit, should be placed in Row 3 corresponding to each conjugate tube in Row 2. Keep foil pouch sealed when not in use.
8. Place the bottles of Substrate A, B and Stop into the appropriate workstation holes indicated on the diagram along with their corresponding labeled 5.0 mL Combitips. Place the 12.5 mL Combitip labeled "Buffer" into the hole under those for the 5.0 mL tips.
9. One pink positive displacement pipet tip should be placed in the hole in the upper left corner of the workstation for every sample being tested. An additional tip should be placed in this hole for the standard. These tips will be used to perform dilutions and transfer sample to the conjugate tubes.

Ordering Information

Description	Catalogue Number
Ensys PAH Soil Test Kit	7061301
SDI Soil Sample Extraction Kit (with methanol in ampules or bulk)	7061301EA or EB
Ensys/Envirogard Field Soil Lab (Accessory Kit)**	6050400
Differential Photometer (110V)	6000001
Differential Photometer (220V)	6000002
5 mL Combitip for Repeating Pipette (1 each)	6005200
12.5 mL Combitip for Repeating Pipette (1 each)	A00009
50 mL Combitip for Repeating Pipette (1 each)	6005600
Gilson Microman Positive Displacement Pipette Tips- yellow (200/bag)	6030500
Gilson Microman Positive Displacement Pipette Tips – pink (200/bag)	6030600
Ensys/Envirogard Field Soil Lab (Accessory Kit) Rental	6997020
**** To obtain part numbers and pricing for individual items in the Field Soil Lab 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. 1~100 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

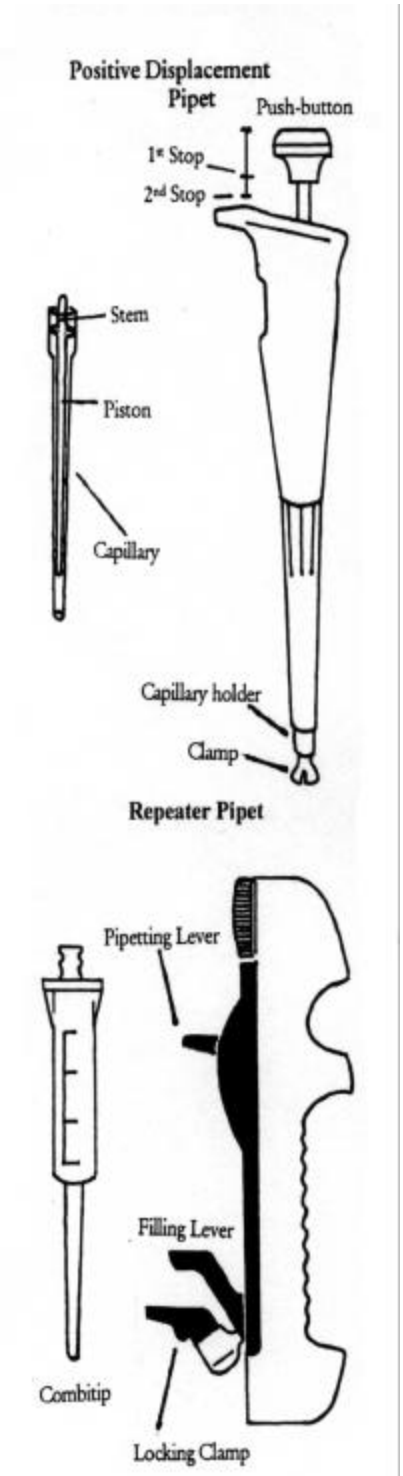
With tip mounted in position on pipet, immerse end of tip into solution. Slide filling lever upward slowly. Combitip will fill with liquid.

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 Positive Displacement Pipet

To Set or Adjust Volume

Turn lower part of push-button to adjust volume up or down. See kit instructions for appropriate setting.

To Assemble Pipet Tip

Press push button to 2nd stop to open clamp (see diagram, this is as far as push button will go down.) Select piston and slide stem fully into clamp. Slide mounted piston into capillary. Gently push capillary until it snaps onto capillary holder.

To Withdraw Sample

With tip mounted in position on pipet, press push-button to 1st stop and hold it. (If you push beyond the 1st stop tip will eject.) 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.

To Dispense Sample

Wipe any liquid from outside of capillary taking care not to touch orifice. Place tip into dispensing vessel (immersing end of the tip if vessel contains liquid) and slowly press push-button to 1st stop. Pipet liquid up and down in tip to ensure complete transfer. Hold push-button at 1st stop when removing tip from vessel.

To Eject Tip

Press push-button to second stop. Tip (capillary and piston) is ejected.

NOTE: When using yellow tips on the positive displacement pipet, pipetting volumes range from 5-25 uL. (i.e. Pipet set on 2-5-0 will pipet 25 uL.)
When using pink tips on the positive displacement pipet, pipetting volumes range from 50-250 uL. (i.e. Pipet set on 2-5-0 will pipet 250 uL.)