

Biochemical Diagnostics, Inc.

Heartland Business Center, 180 Heartland Blvd., Edgewood, NY 11717 • Phone (516) 595-9200

MULTI-PREP® TLC SPOTTER

TABLE OF CONTENTS

- I. SPECIFICATIONS
- II. A. INTRODUCTION
- B. ILLUSTRATIONS
- III. OPERATING INSTRUCTIONS
 - A. INITIAL INSPECTION
 - B. INSTRUMENT TURN ON AND INSPECTION
 - C. INITIAL START UP
 - D. NORMAL START UP
 - E. SPOTTING THE TLC PLATE USING THE SAMPLE APPLICATORS
 - F. SPOTTING THE TLC PLATE USING CAPILLARY PIPETTES
 - G. PRECAUTIONS
- IV. APPLICATIONS
- V. SPOTTING MULTI-PREP COLUMN ELUATES
- VI. TROUBLESHOOTING
- VII. WARRANTY

I. SPECIFICATIONS

1. Power Requirements: 110v+10 10 Amps 50-60 Hz
2. Physical: Single Plate Model (1176)
 - a. Dimensions 10" x 11" x 4"
 - b. Weight: 9 lbs.
3. a. Temperature Accuracy: +/-3%
 - b. Repeatability: +/-3%
4. Temperature Range:
Room Temperature to 60°C on TLC plate surface.
Room Temperature to 99° C on silicone heater (lower side of heating plate temp. measuring point).
5. Environmental: Designed for use at room temperatures from 15°C to 50°C.
6. Cooling: Fan Cooled.
7. Safety:
Automatic shutoff in case of open or shorted heat sensors, overheating or failure of forced airflow.
8. Power Cord: Standard three prong, grounding required.
9. Fuse: 12 amp Resetable Circuit Breaker -110v.

II. A. INTRODUCTION

Biochemical Diagnostics patented, Multi-Prep TLC Spotter is a laboratory instrument designed to quantitatively "spot" column eluates (as they elute from the column) or solvent extracts directly onto regular or pre-channeled TLC plates.

Thin layer chromatography's versatility and powerful separating ability, combined with the potential to run many samples at a time make it an attractive analytical technique in a variety of laboratory situations including toxicology, forensic medicine, clinical chemistry, pharmacology, research and quality control.

The Multi-Prep TLC Spotter enables the user to take full advantage of TLCs' potential.

Multi-Prep Spotters will, with only a few minutes of set up time, simultaneously spot a TLC plate (racks available for spotting 15 or 19 spots per plate) at a rate of 100-150 µL per minute. Sample handling is minimized reducing the chance of error, and sensitivities are increased due to the elimination of transfer losses.

The instrument also facilitates "hand spotting" with capillary pipettes.

III. OPERATING INSTRUCTIONS

A. INITIAL INSPECTION

This instrument has been carefully tested and inspected both electrically and mechanically before shipment. In addition, each unit undergoes a 50 hour electrical burn-in. The instrument should be carefully inspected for damage from transit. The shipping container must be retained for warranty repairs.

B. INSTRUMENT TURN ON AND CHECK OUT

Switch Functions:

Please refer to Fig. 1A for specific locations of controls discussed in this section.

1. Power Switch

The power switch turns on the fan and applies power to the heater.

2. Heat Control

Heat control of the heating plate ranges from ambient to 99°C. The surface temperature of the TLC plate differs because of its insulating characteristics and ranges from ambient to approximately 60°C.

3. Set/Plate Temperature Switch

When in the set temperature mode, this switch is used in conjunction with the heat control switch and the digital display to set the desired operating temperatures. In the plate temperature mode the temperature on the digital display is the temperature of the heating plate.

4. Calibrate

The heat control has its own calibrate screw. When the red L.E.D. display is on, the plate temperature can be calibrated against a surface measuring temperature probe. Units are factory calibrated; therefore these screw settings should normally not be changed.

C. INITIAL STARTUP

1. Turn the heat control switch fully counterclockwise.

2. Place the temperature readout switch in the "SET" position. Turn the heat control knob clockwise until the desired operating temperature is indicated on the digital display.

3. Place the temperature read out switch in the "PLATE" position. When the digital display indicates that the heater is stabilized at the set temperature the instrument is ready to use.

D. NORMAL START UP

The Multi-Prep TLC Spotter will reproduce temperature settings within 3%. For normal start up it is only necessary to turn the heat control fully counterclockwise, turn on the power, set the heater at the desired temperature, and wait for the heaters to come to operating temperature.

E. SPOTTING THE TLC PLATE USING THE SAMPLE APPLICATORS.

1. The Theory of Operation

Multi-Prep TLC spotter consists of two basic components:

- a. The instrument with heater and forced air (previously described) and the sample applicators with accessory applicator and column racks.
- b. Spot size is determined by a combination of heat and air to control the rate of evaporation of samples that are slowly applied to the surface of the TLC plate via a sample applicator containing a microparticle wicking agent and water absorbing filter.
- c. The standard wicking matrix is composed of specially prepared material, which is hydrophilic, physiologically inert, and extremely resistant to all commonly used organic solvents.

Note: Polar solvents such as Methanol:Water mixtures may cause the matrix to swell, slowing solvent flow. The hydrophilic wicking matrix and filter combine to separate trace amounts of water from the solvent; an important feature which helps eliminate non-specific water soluble pigments from depositing onto the TLC plate.

2. General Information

- a. The first order of business is selecting the proper spotting temperature for the particular solvent/solute sample being analyzed. Most applications will probably fall within the 60°C-90°C range.
- b. Place a TLC plate onto the Multi-Prep Spotter and set the heater temperatures to 80°C. Place the sample applicator rack into position over the plate using the locator pins as a guide, (Fig. 2A) and place three or more sample applicators into the rack.

Note: If necessary the sample applicator may be tapped with fingertip to remove gaps which may develop in the matrix bed during shipping. If a slight gap develops between the upper filter and matrix gently push the filter back into place using a wood applicator stick. Make sure that tips are in contact with the TLC plate surface. Poor contact may cause erratic spotting. (See Troubleshooting Section)

- c. Pipet up to 2 mL* of the solvent to be spotted into the applicator and monitor the spot sizes. The spot sizes may now be adjusted by carefully increasing the temperature to reduce spot sizes or decreasing the temperature to increase spot sizes. The temperatures arrived at by this technique will be close to the actual operating conditions used for the samples. Moisture content, solute composition, room temperature and relative humidity, may affect the spot size and necessitate some additional fine-tuning. *

Note: The capacity of the sample applicator is 2.0 mL. 2.0 mL should be added slowly to allow matrix to absorb some of the solution. Rapid addition may cause the solvent to overflow. If the solvent volumes exceed this capacity then the sample must be introduced in aliquots.

CAUTION: If the temperature is raised too quickly above the 90°C setting there is a danger of cracking the glass plates. Plastic sheets may be used, however they should be used at lower temperatures to minimize warping and should be weighted down behind the sample applicator rack to help prevent the plates from lifting if warping does occur. Air gaps between the hot plate and TLC plate (glass or plastic) tend to cut down on heat transfer to the TLC plate causing an increase in spot size.

3. Sample Application

- a. The Multi-Prep TLC Spotter is designed for three different modes of operation. Pipetting of up to 2.0 mL of extraction solvents into the sample applicator; application of column eluates directly from the column into the sample applicator; or adsorbance of up to 200 µL of solvent through the bottom of the sample applicator followed by elution using approximately 500 µL of solvent.
- b. The pipetting of sample directly into the applicator is performed as previously described in Para. 2C.
- c. When going directly from column to applicator it is first necessary to place the column rack into position (Fig. 2B) over the applicator rack using the locator pins as a guide. Racks are held in place using thumb-screws to locate and hold racks in place. Columns are then dropped into position over the applicators and up to 2 mL of the elution solvent is pipetted directly into the column reservoir.

F. SPOTTING THE TLC PLATE USING CAPILLARY PIPETTES.

1. Place a TLC plate onto the Multi-Prep Spotter and set the heater temperature to 80°C. Place the sample applicator rack into position over the plate using the thumbscrews as a guide.
2. Load the sample applicator rack with 1 mL (blue) disposable pipette tips that have been cut back at the tip end to enlarge the opening enough to allow the capillary pipette to freely pass through to the TLC plate. These modified pipette tips will act as a guide for capillary pipettes during the hand spotting process.
3. Proceed with hand spotting by picking up liquid in a capillary pipette and dropping the loaded capillary into the first cutoff pipette tip. Repeat this step for all samples to be spotted on the plate; return to the first capillary and repeat the process until the desired sample volumes have been spotted.

G. PRECAUTIONS

1. The Multi-Prep Spotter should be operated in a fume hood or laboratory room designed for efficient fume removal.
2. Prolonged contact of the spotter housing or column/applicator racks with a solvent can soften the paint. To prevent softening, solvent spills should be blotted dry immediately.
3. There is a sealed pan beneath each heater to isolate the electronics from spilled solvents. The large vents along the sides of each heater plate are to prevent the buildup of vapor pressure under the plate, however for safety reasons, in case of a spill, it is important to shut off the unit, remove the heating plate(s) (4 screws), and wipe the solvent from the pan.

IV. APPLICATIONS

A. DRUGS OF ABUSE MASS SCREENING

The Multi-Prep TLC Spotter and column extraction system have been designed to complement each other. Two Multi-Prep TLC Spotters will handle the drug extraction output of a 28 place workstation.

B. EMERGENCY ROOM TOXICOLOGY

1. The Multi-Prep column extraction system will extract up to 28 samples in fifteen minutes with less than five minutes labor.
2. TLC spotting is completed in approximately 15 minutes by Multi-Prep, yet requires only a few minutes labor.
3. The Davidow solvent system takes approximately 30 minutes to run; other "custom" TLC solvent systems run in less than 15 minutes.
4. Drug visualization takes an additional 10 minutes.
5. Total time is approximately one hour with less than 15 minutes of actual working time.

C. RESEARCH

The large selection of columns available from Biochemical Diagnostics combined with the flexibility of the Multi-Prep TLC Spotter and extraction system make these products ideal research tools. In addition to the standard operations already described, the following options are available:

1. The option to collect column eluates into 15 x 85 mm disposable tubes with subsequent aliquoting of the sample for multiple analysis by techniques such as TLC, HPLC, GC/MS, immunoassays, etc.
2. Collection of column eluates followed by sample dry-down using the Multi-Prep sample evaporator.
3. Splitting of column eluates onto two separate TLC plates using a single sample applicator tip.

D. QUALITY CONTROL

The rapid turnaround time, flexibility and high capacity, combined with quantitative TLC spotting make Multi-Prep a useful addition to the quality control laboratory.

V. SPOTTING MULTI-PREP MINI-BED DRUG EXTRACTION COLUMN ELUATES

After aspiration (See: Multi-Prep Manual) the columns are transferred to the Multi-Prep TLC column racks.

1.5 mL of Multi-Prep Elution Solvent will give drug recoveries comparable to most hand spotting operations where a significant residue remains in the sample tube. If a quantitative transfer technique has been used for manual spotting then it may be necessary to increase the solvent volume to 2.0 mL in order to obtain identical results. Please refer to the Multi-Prep Drug Identification Manual for additional information.

VI. TROUBLESHOOTING

Proper column aspiration is particularly important when using the Multi-Prep Spotter because a poor aspiration may cause some columns to elute very slowly or not at all. If all columns are not dripping freely after two minutes it may be necessary to use the flow inducer to start them going. Too much water in the eluent will also slow down the spotting rate. Up to 100 μ L of water are absorbed by the sample applicator tip without significantly affecting spotting rates or quality.

The proper heating plate temperature for spotting the Ethyl Acetate/Isopropanol Elution Solvent is approximately 75°C. Too high a temperature results in erratic solvent flow through the sample applicator tip and tiny "spots" which may cause sample streaking. Spotting temperatures set too low will result in large spots, which may be unsuitable for routine TLC work.

Make sure that the sample applicator tip is making good contact with the TLC plate. Poor contact will often result in slow and erratic spotting.

If the sample applicator tips are not dropping freely onto the TLC plates during the loading process check for excess paint on the lower set of holes, particularly on the 19 plate sample applicator racks. Check that the rack has not been bent during shipment. This is another possible cause for tips not making good contact with the TLC plate. Occasionally tips may develop air pockets that slow down or block the solvent flow. Lifting the tip momentarily from the plate and tapping it sharply should cause the solvent to begin flowing normally.

VII. WARRANTY

The manufacturer warrants that this instrument has been tested by quality control to insure optimum operating performance and repeatability of results. It further warrants that this instrument is free from defects in materials and workmanship. Should defects in materials or workmanship develop within twelve months from delivery of the product, the manufacturer will service or repair the equipment without charge.

If the manufacturer determines that a fault has been caused by misuse, abnormal operating conditions, or repairs of unauthorized personnel during the warranty period, repairs by the manufacturer will be billed at nominal charge.

The manufacturer reserves the right to perform warranty service either in its own factory or at an authorized repair station. All repairs and replacements of parts under the above warranties are F.O.B. factory or service branch.

The above paragraph does not apply to spotter tips or other accessories.

IIB. ILLUSTRATIONS

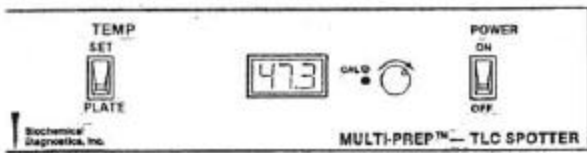


Figure 1A

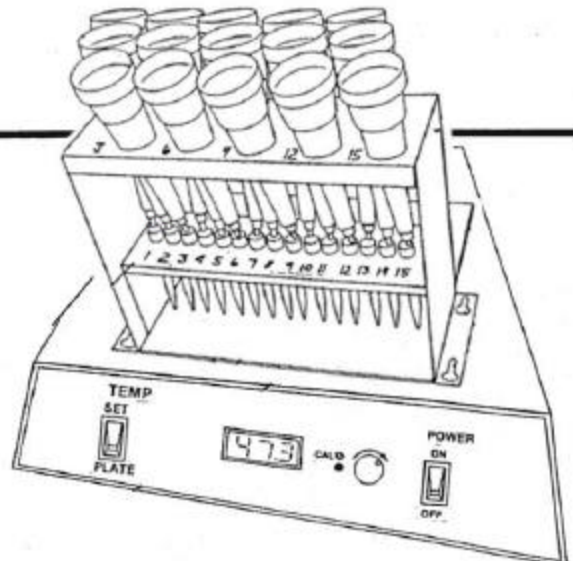


Figure 1B

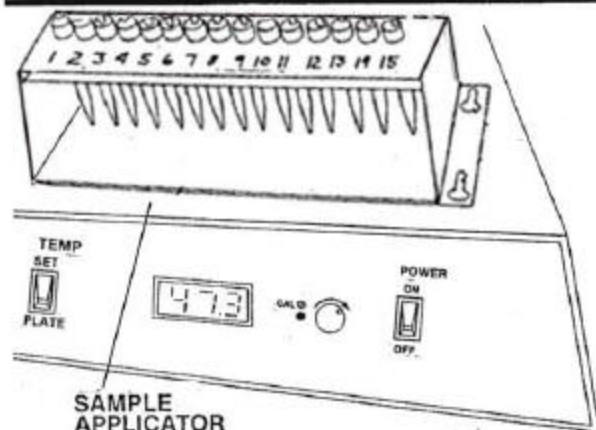


Figure 2A

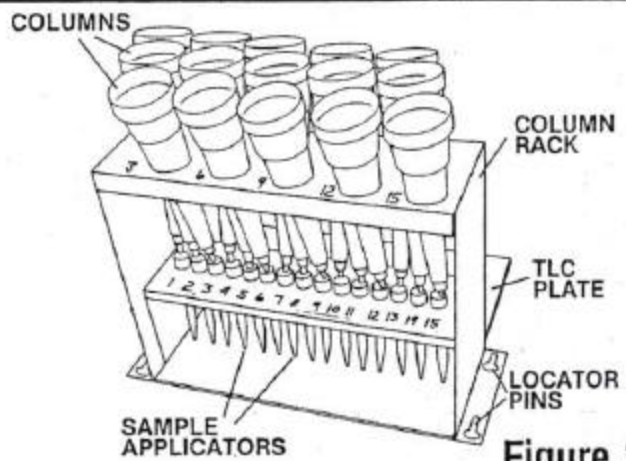


Figure 2B