

# Helena Laboratories

## Operator's Manual



## ProtoFluor-Z Hematofluorometer

Cat. No. 2005, 110 Vac

Cat. No. 2006, 220 Vac

# **ProtoFluor-Z**

Hematofluorometer

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Cat. No. 2005, 100 Vac  
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## ONE – Instrument Use and Function

The ProtoFluor-Z (Fig. 1-1) is a front-face fluorometer designed for the measurement of zinc protoporphyrin (ZPP) in whole blood. This measurement is used as a diagnostic test for relative iron deficiency erythropoiesis.<sup>1-4</sup> The ProtoFluor-Z is intended for in-vitro diagnosis use only.

Heme is formed in the developing red blood cell by insertion of iron into a formed porphyrin ring. In the event of insufficient iron supply (iron deficiency), zinc is substituted for iron and inserted into the porphyrin ring. The ZPP formed in the chelation process is stable and remains in the red cell for its 120 day life span. The level of ZPP in the red cell, then, is a functional indicator of the available iron supply.

The presence of elevated blood lead levels also results in increased ZPP. The CDC has lowered the recommended lead screening level in children from 25 ug/dL to 10 ug/dL. Lead levels below 25 ug/dL do not significantly affect ZPP formation, and therefore the use of this test as a lead screen is no longer recommended. Lamola and Yamane showed that the fluorescent erythrocyte and porphyrin associated with lead intoxication is zinc

protoporphyrin (ZPP) and determined its absorption and fluorescence can be detected easily in whole blood by use of a front face fluorometer.<sup>6,7</sup> The absorption (424 nm Soret band) and fluorescence maxima (595 nm) of ZPP in blood differ from those of the metal-free porphyrins associated with various porphyrias. The fluorescent porphyrin associated with iron deficiency anemia is ZPP which is identical to that produced by elevated lead intoxication.

Blood porphyrin assays other than hematofluorometry involve extraction of the porphyrin(s) followed by some type of purification step.<sup>8</sup> Hematofluorometry using the ProtoFluor Reagent and ProtoFluor Calibrators provides a very simple, easy, accurate and inexpensive method for determining erythrocyte ZPP/H levels in the diagnosis of iron deficiency. Diurnal variation serum iron concentration does not interfere. The method requires only one drop of whole blood and no sample measurements are necessary. In addition to general laboratory diagnosis and nutritional monitoring, the test can be beneficial in specialties such as blood banking, sports medicine, obstetrics and pediatrics where iron status is a particular concern.



**Figure 1-1 ProtFluor-Z**

## TWO – Principles of Operation

The functional units of the ProtoFluor-Z are shown in figure 2-1. All user input is through the external panel controls and the on/off switch. Light from a quartz-halogen lamp is collected and filtered by a lens/filter system to produce light at 415 nm. The 415 nm light is focused onto a sample, held on the sample holder. Part of the 415 nm light is also detected by a photocell. The photocell measurement allows correction for variations in the intensity of the 415 nm light.

When the sample is exposed to 415 nm light, zinc protoporphyrin (ZPP) is excited and emits light at 595 nm. A second lens/filter system collects, filters and focuses the 595 nm light onto a photomultiplier tube (PMT). The PMT produces a current level in response to the light reaching it, which is proportional to the ZPP/H ratio. In the five seconds after pressing MEASURE, over 1000 light-level readings are taken and averaged by the microcomputer, and a value is displayed in  $\mu\text{mol ZPP/mol Heme}$ .

If the user selects the mode for  $\mu\text{g ZPP/dL whole blood}$ , the result in  $\mu\text{mol ZPP/mol Heme}$  is converted by the instrument and the result displayed in  $\mu\text{g ZPP/dL whole blood}$ .

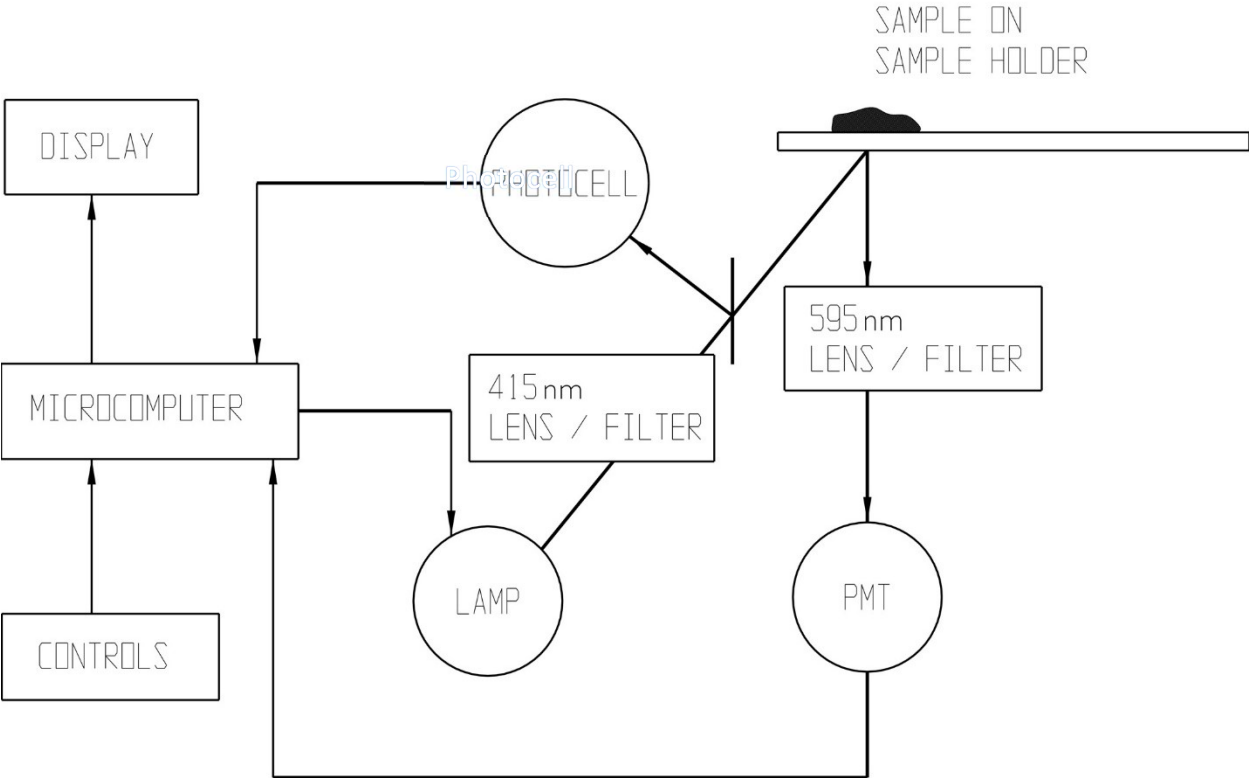


Figure 2-1 Block Diagram

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**THREE – Precautions and Limitations**

3.1. The entire operator's manual should be read and understood before attempting instrument operation.

3.2. Installation is to be performed by the operator.

3.3. Provide adequate room at the sides and back of the instrument for good air circulation.

3.4. Should instruments be contaminated by blood or blood derivatives, spray commercial virucidal and germicidal agent onto the area contaminated. Observe where specimens are used inside the instrument, and confine cleaning to that area. Wipe up the agent residue, as these materials may contain alcohol, which is corrosive to metal surfaces.

No harsh cleansers, acids, or bases should be used or spilled on inner or outer surfaces. Do not immerse the unit.

**ALWAYS TURN THE POWER SWITCH OFF AND UNPLUG THE MAIN POWER CORD BEFORE CLEANING.**

3.5. For emergency shut down, disconnect the power cord or use the power on/off switch located on the rear of the instrument.

**3.6. Do not use hemolyzed specimens. Hemolyzed specimens generate unpredictable results.**

3.7. Do not use frozen specimens or specimens that have been stored longer than recommended.

**3.8. Specimens must be run with reagent.** Results with fresh samples

may be significantly decreased when run without reagent and moderately aged samples run without reagent may generate unpredictable results.

3.9. Abnormally elevated bilirubin will create positive interference due to its spectral qualities.

3.10. Riboflavin may cause elevated results if greater than 10 times normal serum concentration.

3.11. The reagent/sample mixture must be read within 5 minutes of preparation. Make only one reading from each prepared slide. If a repeat reading is necessary, put a fresh drop of sample mixture on a clean coverslip for a repeat reading. Irradiation of the sample may cause photodecomposition.

3.12. Blood samples, calibrators and reagents must be warmed to room temperature before use, since fluorescence is affected by temperature.

3.13. The sample applied to the coverslip must make a spot at least 8-10 mm in diameter in the center of a coverslip.

3.14. Testing should be performed only with Helena coverslips which have been tested for potential fluorescent interference.

3.15. Keep the sample holder level when in use.

3.16. Do not measure sample containing a bubble, since this will cause a falsely low reading.



3.17. The sample holder should be stored in the slot to prevent dust from entering the instrument.

**3.18. The ProtoFluor Z must have annual preventative maintenance, including replacement of the optics. Failure to do so may lead to decreased results.**

Preventative maintenance must occur at Helena Laboratories.

**Results may be affected prior to detection by calibration and optical checks.**

3.19. Should biological or radiological contamination take place, use appropriate decontamination procedures. Should servicing be necessary, the unit must be properly decontaminated and cleaned before servicing will be performed. A written statement (outlining the procedure used) may be required to certify proper decontamination.

3.20. The CDC has lowered the recommended lead screening level in children from 25 ug/dL to 10 ug/dL. Lead levels below 25 ug/dL to 10 ug/dL. Lead levels below 25 ug/dL do not significantly affect ZPP formation, and therefore the use of this test as a lead screen is no longer recommended.

**3.21. The use of ZPP testing for occupational lead exposure testing has not been validated on ProtoFluor Z.**

3.22. Instructions for the “responsible body\*” (\*Under IEC 61010-2-101:2002 – the person(s) responsible for the use and

maintenance of equipment and for ensuring that operators and adequately trained for eliminating and reducing hazards involved in removal from use, transportation, or disposal.)

3.23. Action(s) to be taken in case of malfunction: See section 3.6 and 10.2

3.24. Requirements for handling biohazards: Due to potential biohazard risk from human based components blood, CSF [Cerebrospinal Fluid], urine, plasma, blood cells, etc.), guidelines pertaining to Universal Precautions shall be adhered to when handling the samples and operating this instrument. This includes the use of protective gloves and any other protective equipment as warranted for safe handling and disposal of test tubes, reagents, applicators, or other items containing or contaminated by biohazards and use, transportation and disposal of this device. For information on minimizing biohazard risk, see to section 3.4.

3.25. Storage and transport environmental requirements: Operating Temperature range: 15° to 30° C. Storage and shipping temperatures: -20° to 70° C.

3.26. The Helena Agent shall provide a power cord or adapter of the proper configuration for the country in which the instrument is to be installed. The power cord or adapter will comply with IEC 60227, IEC 60245, or be certified as rated for the power specified if section 9 of this manual.

**FOUR – Hazards**

4.1. This device contains high voltages which can be extremely dangerous. Turn off the power, disconnect the power cord, and use extreme care when attempting disassembly for cleaning, repair, or adjustments. Do not operate any instrument with the cover removed unless instructed to do so by a qualified service technician directly representing Helena Laboratories, its subsidiaries or its distributors.

4.2. Do not attempt to operate the instrument without plugging the power cord into an easily accessible grounded wall outlet of the proper voltage and frequency. This information is contained on the serial number plate located on the back of the instrument.

4.3. Before turning on the instrument power, ensure that the voltage select switch on the rear panel is set for the correct voltage.

## FIVE – Controls and Displays

### 5.1. Controls

**BLANK/SCALE DOWN Button:** Press BLANK once to blank cover slip. When holding the PRESS TO SCALE button down during calibration, press SCALE DOWN to decrease the reading.

**PRESS TO SCALE Button:** During calibration, hold down PRESS TO SCALE and press SCALE UP or SCALE DOWN button to adjust reading to published value for calibrator.

**MEASURE/SCALE UP Button:** Press MEASURE once to read sample. When holding the PRESS TO SCALE button down during calibration, press SCALE UP to increase the reading.

**Power On/Off Switch:** Located on the back panel. Controls power to the instrument.

**Voltage Switch:** Factory preset for 110 Vac or 220 Vac for domestic or foreign use.

**Switch to Select Reporting Units:** Located on the back panel, switch has three positions to select reporting units in umol ZPP/mol Heme, ug ZPP/dL whole blood (25 hematocrit) or ug ZPP/dL whole blood (42 hematocrit).

- 1 (up) ug/dL using 42 Hct
- 2 (middle) umol/mol
- 3 (down) ug/dL using 35 Hct

The mode selected is indicated on the LED display when the MEASURE button is pressed. In the 5 second delay before the ZPP value is shown, the LED display will display the following.

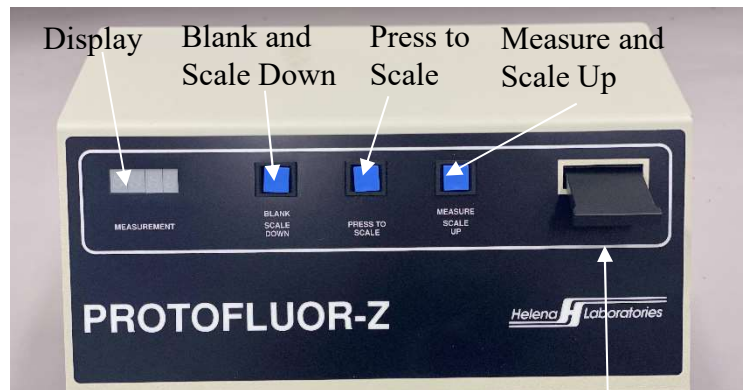
----: in umol/mol mode

H-35: in ug/dL mode, 35 hematocrit

H-42: in ug/dL mode, 42 hematocrit

### 5.2. Display

LED display shows results of measurements in umol/mol or in ug/dL.



Sample Holder

**Figure 5-1. Front Panel Controls and Display**

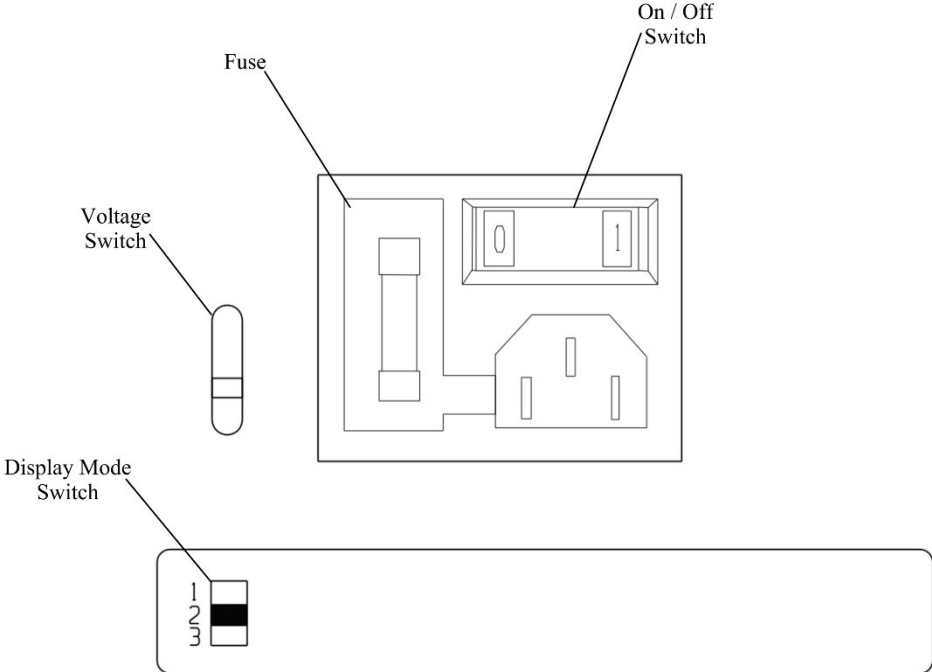


Figure 5-2. Back Panel Controls

**SIX – Installation Instructions**

**WARNING: Read Section Three (Precautions and Limitations) and Section Four (Hazards) before attempting installation or operation.**

Installation is to be performed by the purchaser.

**6.1. Unpacking and Inspection**

1. Check all shipping containers for signs of damage. If damage is found, immediately notify the shipping carrier.
2. Carefully unpack the instrument and accessories and remove them from the shipping cartons. The packing material should be removed undamaged, if possible, should repacking be necessary.
3. Remove plastic wrappings from the instrument and accessories. If scissors or a knife are used to cut the plastic or binding tape, take care not to scratch the instrument.
4. Inspect the instrument for any obvious signs of damage. If damage is found, notify the shipping carrier and Helena Laboratories.
5. Inventory all items: If any parts are missing, recheck the packing materials before notifying Helena Laboratories.

**Table 6-1 Inventory**

1	ProtoFluor-Z
1	Sample Holder
1	Power Cord
1	Operator's Manual

1 Bulb Assembly (for microhematocrit tube)

**6.2. Installation**

1. Select an environment free of drafts, excessive humidity, dust, and large temperature fluctuations.
2. Place the unit on a level, flat surface. Make sure that there is enough space behind and around it to allow good air circulation.
3. Insert the sample holder into the slit at the upper right marked SAMPLE. The end with the hole should enter the instrument with the recessed area facing up. Push it into the slot until it clicks into place.
4. Ensure that the voltage switch on the back panel is set for the correct input voltage (115 V for 110 V unit or 230 V for 220 V unit). If incorrect, change the switch position using a small screwdriver and see section 10.1.1 (Fuse Replacement) for proper fuse selection.
5. With the power off, insert the female end of the power cord into the three-prong plug on the back of the instrument.
6. Plug the other end of the power cord into a grounded wall outlet of the proper voltage and frequency. Because the power cord is the mains disconnect device, the wall outlet used should be easily accessible. This information is located on the serial number plate on the back panel of the instrument.

The wall outlet should not be on the same circuit as any large load device such as a refrigerator, compressor, centrifuge, etc.

The instrument's circuitry contains filters to reduce the effect of line voltage fluctuations: however, they should still be avoided. If the operator experiences difficulty, it may be necessary to install an isolation transformer.

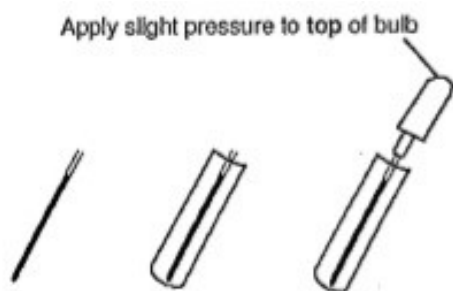
## SEVEN – Operating Instructions

### 7.1 Specimen Collection and Handling

**Specimen:** Whole blood collected with an anticoagulant such as heparin, EDTA, or citrate.

**Blood collected from a fingerstick:**

Collect a drop of blood from a fingerstick into a heparinized microhematocrit tube. The capillary tube must have a volume of at least 50  $\mu$ L. Fill the capillary tube to the line. Drop the capillary tube into a 13x75 mm test tube. Expel all the blood from the capillary tube using the bulb assembly supplied with the instrument. Add 2 drops of ProtoFluor Reagent and proceed with the test.



If the test is not to be run immediately, the capillary tube can be sealed at both ends with Critoseal and stored. When ready to perform the test, cut off the ends and expel the blood into the test tube.

**Patient preparation:** No special patient preparation is required.

**Interfering factors:**

1. **Do not use hemolyzed specimens.** Hemolysis is indicated when the plasma has a reddish color due to the rupture of red blood cell membranes. Unreliable

results (Incorrectly low or high) are obtained with hemolyzed specimens.

2. **Samples stored longer than recommended may have falsely increased or decreased results.**

3. Abnormally elevated bilirubin will create positive interference (high values) due to its spectral qualities. Increased bilirubin is indicated by a bright yellow color in the plasma.

4. Riboflavin concentrations greater than 10 times normal may cause elevated results. Patients receiving nutritional therapy may have increased levels of riboflavin.

5. **Routine washing of cells to eliminate plasma interferences is not recommended.**

6. Freezing of specimens, hemolysis, failure to use reagent, and failure to maintain instruments may cause falsely low results.

**NOTE:** Even though there are a few interferants that may cause falsely elevated ZPP values, extensive case studies have shown that any abnormal result obtained with zinc protoporphyrin measurements is indicative of some pathological condition requiring intervention.

**Stability of sample preparation:** After adding one drop of well mixed whole blood to two drops of reagent, the sample mixture should be used within five minutes.

**Storage and stability:** Specimens should be analyzed soon after collection and before hemolysis occurs. **Storage at 4°C for up to one week may be acceptable, but as anticoagulated blood ages, it becomes hemolyzed, which may cause erroneous results.**

### 7.2. Preparation and Warm-up

1. Verify that the instrument is in a location free of a draft from air conditioning or heating vents.

Drafts from heating or air conditioning vents result in fluctuating values which are particularly noticed when trying to calibrate the instrument

2. Turn on the power if unit is off. It is recommended that the unit be left on when not in use and only cycled off/on prior to beginning test.

**NOTE: If your instrument is left on continuously, turn the power off and on again to reset electronics.**

The instrument can be left on continuously, if preferred. However, instruments left on continuously must have the power turned off and on again to clear all previous settings before a new daily setup

3. Verify that the instrument is in the correct mode for the units to be reported. Properly set switch on back of unit.

Position	Mode
1 (Up)	ug/dL (42 Hct)
2 (Mid)	umol/mol
3 (Down)	ug/dL (35 Hct)

4. Allow the instrument to warm-up at least 30 minutes.

This 30 minute warm-up warms the electronics in the instrument, but the light bulb is on only when MEASURE is pressed.

5. Press MEASURE 5 to 10 times to warm up the light bulb, waiting approximately 5 seconds between each time the button is pressed for the screen to reset. This cycles the light bulb on and off and should be done prior to making the first reading.

6. Remove calibrators and samples from the refrigerator and allow to equilibrate to room temperature before testing begins (requires about 30-45 minutes). ProtoFluor Reagent is stored at room temperature.

Fluorescence is affected by temperature and inaccurate readings will be obtained if samples and reagents have not equilibrated to room temperature.

### 7.3. Blank Instrument

1. Insert the empty sample holder (no coverslip) into the instrument.

When the sample holder is completely inserted into the instrument, a “click” is heard as the sample holder is pushed into place.

2. Press MEASURE.

Note that after MEASURE is pressed, the display will read either (----), H35 or H42 (indicating the mode of operation) for 5 seconds and then a value appears.

The reading on the blank cavity (sample holder inserted with no coverslip) should be 000-003. Higher values may indicate



a problem. Call Helena Electronics Customer Services Department for assistance.

The instrument requires 5 sec. to recover from a reading. Therefore, it will not accept a new command for 5 seconds after a reading appears on the LED display.

3. Place a clean lint-free coverslip into the sample holder.

Handle coverslips with care to avoid lint, fingerprints and scratches. Be sure multiple coverslips are not stuck together.

Any time a result is obtained that seems out of line, such as duplicate samples with very different readings, the first suspect should be that 2 coverslips are stuck together or that a coverslip was dirty.

4. Insert the sample holder into the instrument.

Be sure you feel the “click” indicating the sample holder is completely inserted.

5. Press BLANK.

The number that appears on the LED display is the value of the coverslip that will be subtracted from the patient reading. An acceptable value is 4-14 for instruments with a serial number beginning with 0, or 7-40 for instruments with a serial number beginning with 2. If the reading is outside this range, obtain another coverslip, insert it into the instrument, and press BLANK again.

6. Press MEASURE.

The coverslip should now measure 000 +/-1 indicating that the unit is zeroed on the coverslip.

**Note:** *If 000 +/- 1 is not achieved, repeat the process with a new coverslip. If the new coverslip fails, contact Helena Laboratories.*

#### 7.4. Calibrate Instrument

1. Withdraw the sample holder from the instrument.

2. Place a coverslip in the sample holder and insert into the instrument.

Use the precautions noted in 7.3 for correctly handling coverslips

3. Press BLANK.

4. Remove the sample holder with blanked coverslip from the instrument and place one drop of ProtoFluor High Calibrator in the center of the coverslip.

The volume of the Calibrator on the coverslip should be enough to make a spot at least 8 to 10 mm in diameter in the center of the coverslip. Covering the entire opening in the sample holder is not necessary.

Do not use a sample preparation containing a bubble, since this will cause falsely low readings.

5. Gently insert the sample holder into the ProtoFluor Z and press MEASURE.

This initial reading of the calibrator may be as much as +/-30 of the published value.

6. Set the instrument to the published value of the Calibrator. **Use the appropriate units of measure.**

The Calibrator value is printed on the assay card in the kit. You will note that three values are provided on the card. Use the value applicable to the mode you are using.

Hold down the PRESS TO SCALE button continuously and press either SCALE UP or SCALE DOWN until the published value appears on the LED.

The instrument is calibrated by pressing two buttons on the front panel of the instrument. Always press the SCALE button first, then while continuing to hold it down, press either the SCALE UP or SCALE DOWN button to set the calibrator to the appropriate value.

7. Withdraw the sample holder and discard the used High Calibrator preparation. Place a new coverslip in the sample holder, insert it into the instrument, and press BLANK.

8. Using the blanked coverslip, insert another preparation of High Calibrator, insert it in the instrument, and press MEASURE.

The reading should be within +/-15 of the published value of the High Calibrator. If not, set the Calibration again as instructed in step 6.

9. Withdraw the sample holder and discard the used coverslip. Place a new coverslip in the sample holder, insert it into the instrument and press BLANK.

10. Remove the sample holder with the blanked coverslip from the instrument

and place one drop of ProtoFluor Low Calibrator on it and insert it into the instrument.

11. Press MEASURE.

The reading of the low calibrator should be within +/- 3 (+/-6 for position 2 (umol/mol)) of the published value. If this reading is not within range, press MEASURE 2 to 3 times. If 2 out of 3 readings are within +/-3 (+/-6 for position 2 (umol/mol)) of the published value, the instrument is set correctly. If it still does not meet specifications, recalibrate with the high calibrator and verify with the low calibrator. Do not change the calibrator settings using low calibrator.

13. Calibration of the instrument is complete.

### 7.5 Patient Sample Assay

1. Thoroughly mix the patient whole blood sample.

2. Add 1 drop of patient sample to a 12x75mm test tube.

3. Add 2 drops of ProtoFluor Reagent to the tube and mix well with gentle shaking (approximately 2 seconds).

Vortexing is not recommended.

The sample reagent mixture must be used within 5 minutes of preparation.

For neonate or minimum quantity samples, use 50 uL of whole blood and 100 uL of reagent. This is approximately the amount of sample in a microhematocrit tube.

4. Withdraw the sample holder and put a clean, lint-free coverslip on the sample holder.

5. Press BLANK.

6. Within 5 minutes of preparation of the ProtoFluor Reagent, pour the patient sample onto the center of the coverslip, covering an area at least 8 to 10 mm in diameter.

Avoid scratching the coverslip with the lip of the tube, and avoid contamination of the sample holder or interior of the ProtoFluor Z. Do not use sample containing a bubble, since this will cause a falsely low reading.

7. Gently insert the sample holder into the instrument.

8. Press MEASURE.

After a 5 second delay, the ZPP value will appear.

If the display flashes 9999, the ZPP/H ratio is above 600  $\mu\text{mol}$  ZPP/mol Heme, or 270  $\mu\text{g/dL}$  whole blood, and the results should be recorded as greater than 600  $\mu\text{mol}$  ZPP/mol Heme or 270  $\mu\text{g/dL}$  whole blood. Dilution of the sample and re-running is not appropriate and not necessary due to the principle of front-faced fluorometry.

9. Record the result, withdraw the sample holder and discard the coverslip.

10. Prepare and measure the next sample and record the reading.

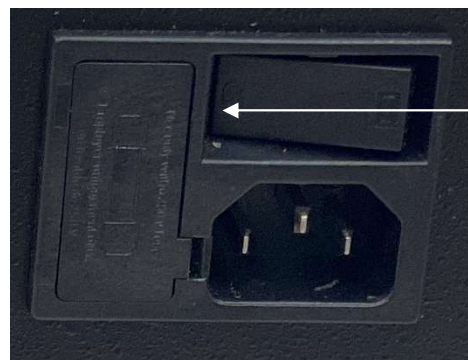
Multiple reagent/sample preparations may be prepared at once as long as they are used with 5 minutes of preparation.

## 7.6 Summary Instructions

**Note:** Before initial operation of the instrument, read the instructions in Sections 7.2 to 7.5 for a complete explanation of the importance of each step. The instructions in this section are condensed for efficiency in using the instrument after thorough understanding of the system.

### Preparation

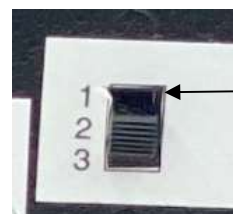
1. Verify that the instrument is in a location free of drafts from air conditioning or heating vents.
2. Turn on the instrument and allow it to warm up 30 minutes.



Turn power on, warm up for 30 minutes

3. Verify that the instrument is in the correct mode for the units to be reported. Properly set the switch on the back of unit:

- 1  $\mu\text{g/dL}$  at 42 Hct
- 2  $\mu\text{mol}$  ZPP/mol Heme
- 3  $\mu\text{g/dL}$  at 35 Hct



Set Mode Switch:

1.  $\mu\text{g}/42$
2.  $\mu\text{mol}$
3.  $\mu\text{g}/35$

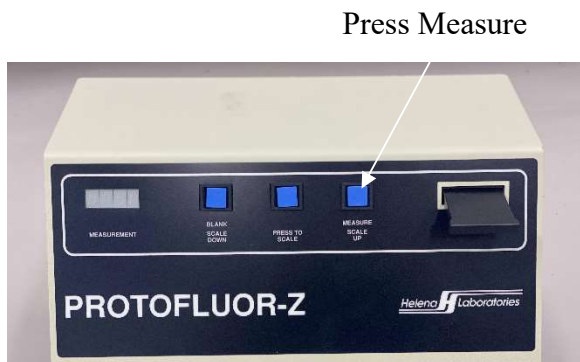
4. Remove all reagents, calibrators, and samples from the refrigerator and allow them to equilibrate to room temperature (about 30-45 minutes).

**Blanking**

1. Insert an empty sample holder into the instrument (no coverslip used).



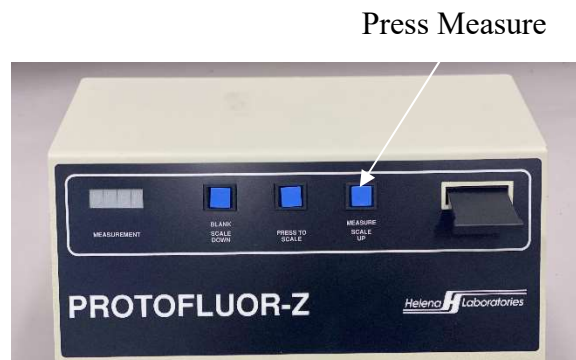
2. Press MEASURE. The LED should read 000-003, indicating that the background reading is correct.



3. Place a clean, lint-free coverslip on the sample holder.

4. Insert the sample holder in the instrument. Press BLANK. The reading should be 4-14 for instruments with serial number starting with 0, or 7-40 for instruments with serial number starting with 2.

5. Press MEASURE. The reading will be 000 +/-1 if the instrument has properly blanked the coverslip.

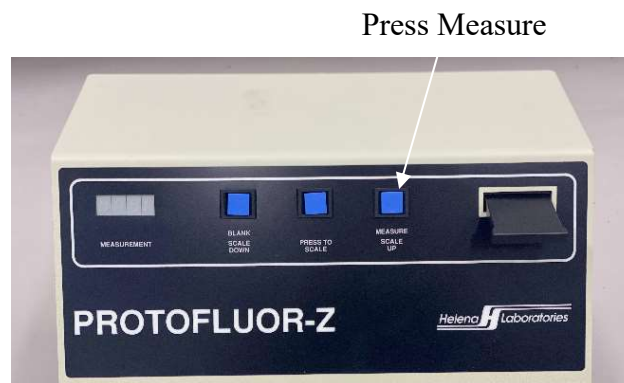


**Calibration**

1. Remove the sample holder from the instrument and leave the coverslip in the sample holder.

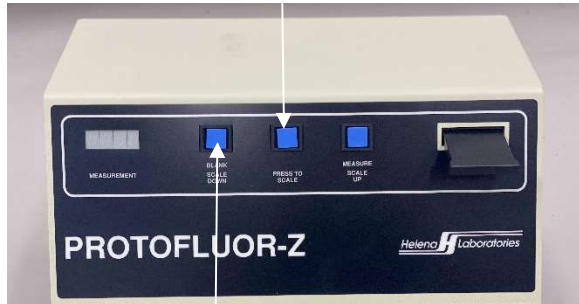
2. Place a drop of ProtoFluor High Calibrator in the center of the coverslip (in the sample holder) covering a spot at least 8-10 mm in diameter (see illustration).

3. Insert the sample holder into the instrument and press MEASURE. The calibrator should read within +/-3 of the value printed on the assay card.



4. Set calibration to the published value by continuously holding down the PRESS TO SCALE button and press either SCALE UP or SCALE DOWN until the published value appears on the LED.

Hold Down Press to Scale Button



Press Scale Down

Always press the SCALE button first, and while holding it down, press either SCALE UP or SCALE DOWN to set the calibrator to the appropriate value.

5. Place a clean coverslip in the sample holder. Insert it into the instrument and press BLANK.

6. Place a fresh drop of high calibrator on the coverslip. Insert it in the instrument and press MEASURE.

The calibrator reading should be within +/-15 of the published value. If not, adjust it once again. After adjustment, repeat Steps 5 and 6 to verify that the instrument is set properly.

7. Place a clean coverslip in the sample holder, insert it in the instrument and press BLANK.

8. Place a drop of ProtoFluor Low Calibrator on the coverslip. Insert it in the instrument and press MEASURE.

The low calibrator should be within +/-3 (+/-6 for position 2 (umol/mol)) of the published value. If the reading is not within this range, press MEASURE 2 to 3 times. If 2 out of 3 of the readings are within +/-3 (+/-6 for position 2 (umol/mol)) of the published value, the instrument is properly calibrated.

If the readings are not satisfactory, the instrument should be reset with the high calibrator. Do not change the settings using low calibrator.

Calibration is complete. The instrument is ready for measuring patient samples.

### Patient Sample Assay

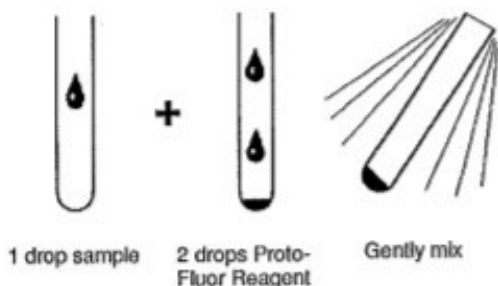
1. Collect the patient sample in a vacutainer tube containing EDTA or heparin, or collect it in a heparinized microhematocrit tube with a volume of at least 50 uL.

See Specimen Collection and Handling for complete instructions on collecting from a finger stick.

2. Put one drop of blood in a 12x75 mm test tube.

3. Put 2 drops of ProtoFluor Reagent in the test tube.

4. Mix the blood and reagent by gently shaking the tube approximately 2 seconds. Do not vortex.



5. Put a clean coverslip on the sample holder. Place the holder in the instrument and press BLANK.

6. Pour the contents of the tube onto the coverslip. This is best done with the sample holder laying on the counter top. Don't hold the sample holder in your hand since this does not provide solid support.



Tap the lip of the tube gently but firmly on the coverslip so that a drop of blood will flow from the tube.

7. Insert the sample holder in the instrument and press MEASURE. After a 5 second delay, the result will appear on the LED. Depending on the mode selected, the value will be in ug/dL whole blood or in umol ZPP/mol Heme.

8. Record the result and discard the used coverslip.



### 7.7. Procedural Notes

1. Be very careful to avoid contamination of the sample holder and the inside of the instrument with blood, calibrators, or control materials. Clean the sample holder thoroughly if it is contaminated with a sample. Contamination of the inside of the optical system will lead to erroneous results and the instrument must be taken apart and cleaned. Be sure to contact the manufacturer prior to opening the instrument.

2. Do not measure a sample that has a bubble in it. Bubbles cause results to read low.

3. Correct reagent to blood ratio is important to the precision of the system. Be consistent with the method of delivery of a drop of blood and two drops of reagent into the mixing tube.

4. The reagent and sample mixture should be read within five minutes after preparation. Values increase after five (5) minutes.

5. Blood samples, calibrators, controls and reagent must be warmed to room temperature before testing begins. Fluorescence is affected by temperature, therefore, equilibrium to room temperature is very important.

6. Make only one reading from each prepared slide. If a repeat reading is necessary, place a fresh sample on a clean coverslip and make a second reading. Irradiation of the blood sample during reading may cause photodecomposition, making subsequent measurements from the sample unreliable.
7. Take care in handling coverslips. Be sure coverslips are free of lint and finger prints. They should always be handled by the edges. In placing and spreading sample on coverslips, be careful not to scratch the coverslip with the test tube. Make sure that multiple coverslips are not stuck together.
8. Do not use hemolyzed specimens. Hemolysis is indicated when the plasma has a reddish color due to the rupture of red blood cell membranes. Unreliable results (incorrectly low or high) are obtained with hemolyzed specimens.
9. Samples stored longer than recommended may have falsely increased or decreased results.
10. Abnormally elevated bilirubin will create positive interference due to its spectral qualities. Elevated bilirubin is indicated when the plasma has a bright yellow (icteric) appearance.
11. Routine washing of cells to eliminate plasma interferences is not recommended.
12. Freezing of specimens, hemolysis, failure to use reagent, and failure to maintain instruments may cause falsely low results
13. When results are reported as a ratio of ZPP/Heme, results are not affected by hemoglobin concentration or hematocrit, eliminating the need to correct for hematocrit.
14. The instrument provides readings from 0-600 umol ZPP/mol Heme. A sample with a ZPP value greater than this or 270 jg/dL whole blood will cause the LED display to flash 9999. The results should be reported as “greater than 600 umol ZPP/mol Heme,” or “greater than 270 ug/dL whole blood” if the concentration mode is being used.
15. Calibration may be verified periodically. Experience with the instrument by verifying the High Calibrator reading periodically during the run and after all samples are tested has shown that the calibration is stable.
16. Sample volume on the coverslip should be adequate to make a spot with a diameter of 8-10 mm. It is not necessary to cover the entire opening in the holder, but the sample must cover the middle.
17. The Protofluor Z can be left continuously on with no deleterious effects. Frequently powering the instrument on and off should be avoided where possible.
18. When use of a minimum blood sample is necessary (neonatal patients), 50 uL whole blood and 100 uL ProtoFluor Reagent is recommended.

## 7.8 Reference Values and Interpretation of Results

Ug ZPP/dL whole blood X ----- = corrected ZPP  
 (Instrument reading) assumed Hct value  
 (35 or 42)

### Diagnosing Iron Deficiency

A reference range study conducted by Helena Laboratories, which included 150 men and women in good health, provided the following normal range for zinc protoporphyrin:

30-80 umol ZPP/ml Heme  
 15-36 ug ZPP/dL whole blood\*

### Interpretation of Results

Iron deficiency, resulting from blood loss or insufficient iron intake, absorption or utilization, may occur during periods of increased need such as rapid growth or pregnancy. ZPP measurements are a means of diagnosing subclinical changes in the biosynthetic pathway of heme before anemia develops. Elevated ZPP may also indicate anemia which may be associated with chronic infection or malignancy.

The presence of elevated blood lead levels also increased ZPP. The CDC has lowered the recommended lead screening level in children from 25 jg/dL to 10 ug/dL. Lead levels below 25 ug/dL do not significantly affect ZPP formation, and therefore the use of this test as a lead screen is no longer recommended.

**The use of ZPP testing for occupational lead exposure testing has not been validated on the Helena system.**

\*When obtaining values in concentration, more accurate results can be obtained by correcting for the patient's actual hematocrit value using the following formula:

Measured Hct



**EIGHT – Test Functions and Quality Control**

The instrument automatically performs a self-test any time the power is turned on. The display should read PFZ. Should an error message appear on the display, turn the instrument off and on again. Should the error message reappear, see Section Ten, Troubleshooting.

Each laboratory should establish its own normal range of expected values for the procedures in use. Refer to Section 7.4 for the calibration procedure.

**NINE – Performance Specification**

**Measurement Range:** 0 to 600 umol  
ZPP/mol Heme

**Input Power:** 110 Vac, 50/60 Hz, 50  
Watts  
220 Vac, 50/60 HZ, 50  
Watts

**Fuses (2):** 3-A/250-V slow blow (110 V  
unit)  
1.6-A/250-V slow blow (220  
V unit)

**Dimensions:** 7-1/2 in. (19 cm) High  
11-5/8 in. (29.5 cm) Wide  
11-1/4 in. (28.6 cm) Deep

**Weight:** 12.6 lb (5.7 kg)

**Environment:** 15° to 30°C (59° to 86°F)

**Performance Characteristics:** CV's of  
less than 5% for all within-run, run-to-run,  
and instrument-to-instrument precision  
tests.

## TEN – Maintenance, Troubleshooting, Warranty

This section describes routine operator maintenance procedures. For instrument calibration or for maintenance not described in this manual, call Helena Laboratories for assistance.

### 10.1. Maintenance

#### 10.1.1. Fuse Replacement

1. Turn off the power and unplug the power cord at both ends.
2. Use a screwdriver blade to withdraw the fuse block (Fig. 10-1).
3. Replace the fuse in the holder (3-A/250-V slow-blow for 110 V unit, 1.6-A/250-V slow blow-blow for 220 V unit).
4. Slide the fuse holder firmly back in place.
5. Replace the power cord, instrument side first.
6. Turn on the power. If the fuse blows immediately, contact Helena Laboratories for assistance.

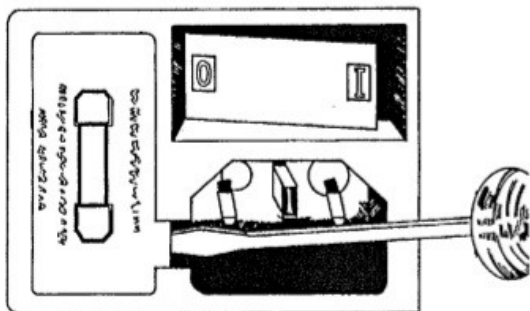


Fig. 10-1 Removing Fuse Block

#### 10.1.2. Annual Preventative Maintenance

**The ProtoFluor Z must have annual preventative maintenance, including replacement of the optics. Failure to do so may lead to decreased results. Preventative maintenance must occur at Helena Laboratories. A decrease in results may occur prior to detection by calibration and optical checks.**

#### 10.1.3. Cleaning Spills

Clean exterior spills with a soft cloth or sponge only after turning off the power and unplugging the power cord. Do not use corrosive or abrasive cleansers. Dry the unit before plugging the power cord in.

NOTE: If liquid enters the instrument, unplug the unit and return it to Helena Laboratories. This is not a user-serviceable feature.

#### 10.1.4. Decontamination

Should biological or radiological contamination take place, use appropriate decontamination procedures. Should servicing be necessary, the unit must be properly decontaminated and cleaned before servicing will be performed. A written statement (outlining the procedure used) may be required to certify proper decontamination.

## 10.2 Troubleshooting

If the recommended solutions should fail to solve a problem, call Helena Laboratories for assistance.

**Table 10-1. Troubleshooting**

<b>Problem</b>	<b>Possible Cause</b>	<b>Solution</b>
No power to instrument When power is turned on	Power cord unplugged	Plug cord into proper wall outlet
	Blown Fuse	Replace fuse
Light inside instrument But no readings	Electrical problem	Contact Helena Laboratories for assistance
After zeroing, no readings	Electrical problem	Contact Helena Laboratories for assistance
Err1, Err2, etc. Displayed at power on	Error found during self-test	Turn power off and on again. If still is displayed, Call Helena Laboratories For assistance
Display contains Decimal points	Error found during operation	Turn power off and on again. If still is displayed, Call Helena Laboratories For assistance
Instrument does not Respond to keys	Electrical problem	Contact Helena Laboratories for Assistance.
Blanked coverslip value Greater than 30	Dirty optics or dirty coverslips	Contact Helena Laboratories for Assistance.
Unable to calibrate	Electrical problem	Contact Helena Laboratories for Assistance.
Unable to calibrate	Instrument insufficiently warmed	Allow to warm longer

Unable to calibrate	Lamp insufficiently warmed	Additional lamp cycles
Patient samples run low	Optics dirty or overdue for maintenance	Contact Helena Laboratories for Assistance
Air blank > 3	Optics dirty or overdue for maintenance	Contact Helena Laboratories for Assistance

### 10.3. Warranty

Helena warrants each new unit to be free from defects in materials and workmanship. Helena's liability under this warranty is limited to servicing and adjusting any unit returned to the factory, servicing and adjusting any unit on site when performed by a Helena service representative, and replacing any defective part. This warranty is effective for 6 months commencing from the date of shipment to the original purchaser and does not cover fuses or faults caused by misuse, abnormal conditions or damage incurred in shipment.

















This warranty is made in lieu of all other warranties, expressed or implied, and is limited in any sale to the repair or replacement of the affected component. It does not cover any accessory-hardware or software purchased by the operator to modify the unit from the original designed application.

### 10.4. Bibliography

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## ELEVEN – Symbology

NOTE: The following symbols may be used in this manual, or on the instrument, to provide information necessary to the user, if applicable.

	Caution, electric shock hazard, high voltages capable of causing personal injury - shut down the instrument and unplug the power cord before touching - do not operate with the cover(s) removed
	Caution, heat hazard - allow heated components to cool before handling
	Caution, general hazard - see Precautions and Hazards (Sections 3 and 4) of Operator's Manual before proceeding
	Direct current
	Alternating current
	Both direct and alternating current
	Ground (earth) terminal
	Protective conductor terminal (grounded conductors)
	Frame or chassis terminal
	Equipotentiality (conductor with all parts at a single potential)
	On (power switch)
	Off (power switch)
	Equipment protected throughout by double insulation or reinforced insulation (equivalent to Class II of IEC 536)
	European authorized representative
	Manufacturer
	Indicates "do not place in trash" in countries or regions requiring recycling and other specific handling, such as in Europe, under the WEEE (Waste Electrical and Electronic Equipment) Directive, 2002/96/EC

ProtoFluor-Z Hematofluorometer  
Operator's Manual

Catalog No. 2005, 110 Vac  
Catalog No. 2006, 220 Vac

For Additional Information or Assistance, call your local distributor, or call Helena Laboratories at 1-800-231-5663 toll free.



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