

### 1 Prepare Reagents

1. Remove Peroxide Reagents A, B, C, Calibrators, Control and Preparation Reagent from the refrigerator and allow them to reach room temperature (18–25°C) This should take approximately 2 hours.
2. Power **ON** the SafTest™ analyzer by pressing the [I] marked portion of the power switch, which is located on the rear panel of the SafTest™ analyzer. Allow 5 minutes to warm up.

### 2 Set up the Calibration Curve

**NOTE:** When you receive a new kit you must first run a calibration curve

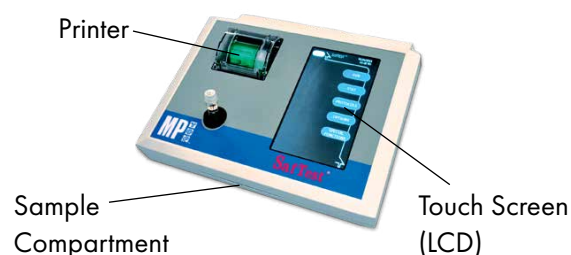
1. Label 4 new 12 mm glass test tubes to correspond to Calibrator 1, 2, 3 and 4; and one new 12 mm tube for the control.
2. Gently swirl the contents of the calibrators and control bottles to ensure you have a homogenous solution.
3. Use a positive displacement pipette to transfer 200 µL of each Calibrator into the corresponding labeled test tube.
4. Use a positive displacement pipette to transfer 200 µL of the Control into the corresponding labeled test tube.

**NOTE:** Use a new tip for each Calibrator and Control. The same tip can be used if running a duplicate. Must remove all air bubbles from tip before drawing final sample

### 3 Add Reagents & Vortex

1. Before dispensing reagents, gently swirl the contents of each reagent bottle.
2. To eliminate air bubbles and oxidized reagent, dispense 4–5 aliquots of each reagent into a waste container immediately before use.
3. Dispense 1 aliquot of Peroxide Reagent A into every calibrator and control test tube.
4. Dispense 1 aliquot of Peroxide Reagent B into every calibrator and control test tube.
5. Dispense 1 aliquot of Peroxide Reagent C into every calibrator and control test tube.
6. Once you have aliquoted Peroxide Reagent C into the last test tube start the timer for 10 minutes.
7. Cap the test tubes and vortex them at the fastest dial setting for 10 seconds.
8. Place the test tubes on the test tube rocker for the remaining time.

**NOTE:** Once time has ended, **IMMEDIATELY** begin reading your samples. **Reading sample at 10 minutes is vital.** Chemical reaction does not stop. If samples are not read immediately, the tubes must be discarded and test should be re-run



## 4 SafTest™ Analyzer Set Up with Calibration [RUN]

1. Press [RUN] icon on the touch screen.
2. Press [PERMSA / PEROXIDE] icon; when highlighted yellow, press [NEXT] icon.



3. At the “Blank Tube” prompt, insert a full test tube of distilled water in the sample compartment. Do not remove tube until prompted or after beep.
4. At the “Cal #\_ of 4” prompts, insert Calibrators 1–4 in the sample compartment.
5. Under the Equ. Tab, verify that the equation of the line meets the following initial guidelines and press [NEXT]

**NOTE:** Please refer to the Control Package Insert

6. If any of these guidelines are not met, recalibrate the instrument.

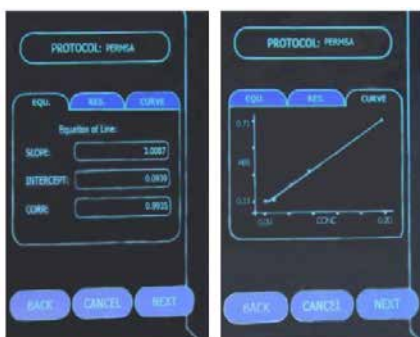


Figure 1.  
PERMSA / PEROXIDE  
calibration screen shots

7. At the “DO YOU WANT TO STORE THE CALIBRATORS” prompt, select YES (Figure 2).



Figure 2.  
PERMSA / PEROXIDE store  
calibrators screen shot

8. Select the number of replicates to be analyzed (1–5). Use the “+” or “-” arrows to select a number (normally duplicates, i.e. 2 samples should be analyzed to ensure results are accurate and consistent).
9. Press the [NEXT] icon.
10. At the “Smpl #\_ Rep #\_ Insert Tube” prompt, insert the duplicate Control tubes into the sample compartment.
11. SafTest™ analyzer will continue asking for samples until the user quits the program by pressing [STOP] and then [DONE].

**NOTE:** The SafTest Analyzer arbitrarily numbers each sample 1–99. Be sure to re-label the sample you are running

12. Verify that the Control falls within the Peroxide Control range which can be found on the Peroxide Control Package Insert. Your test value for the Control should approximate this range. If it does not, rerun the test.

### 5 Prepare Samples

1. Samples should be prepared according to **Sample Preparation Quick Card**.
2. Label one new 12 mm glass test tube per sample or two new glass test tubes if preparing duplicates.
3. Read each test tube twice when prompted for a duplicate. When analyzing duplicate samples, ensure each test tube is read a total of two times. A sample, when run in duplicate, should have four total readings (two each for each test tube).
4. Use a positive displacement pipette to transfer 200  $\mu$ L of each prepared sample into the corresponding labeled test tube.
5. Dispense 1 aliquot of Peroxide Reagent A into every sample test tube.
6. Dispense 1 aliquot of Peroxide Reagent B into every sample test tube.
7. Dispense 1 aliquot of Peroxide Reagent C into every sample test tube.
8. Once you have aliquoted Peroxide Reagent C into the last test tube start the timer for 10 minutes.
9. Cap the test tubes and vortex them at the fastest dial setting for 10 seconds.
10. Place the test tubes on the test tube rocker for the remaining time.

### 6 Analyze Samples

1. Press **[STAT]** icon (Figure 3).



Figure 3.  
Home Screen Shot

2. Press **[PERMSA / PEROXIDE]** icon, when highlighted yellow press **[NEXT]** icon.
3. At the “Blank Tube” prompt, insert a test tube of distilled water in the sample compartment.
4. Select the number of replicates to be analyzed. Use the “+” or “-” arrows to select a number (Normally two replicates should be analyzed to ensure that results are consistent).
5. Press **[NEXT]** icon.
6. At the “Smpl #\_ Rep #\_, Insert Tube” prompt, insert control or sample tubes in the sample compartment.
7. SafTest™ analyzer will continue asking for samples until the user quits the program by pressing **[STOP]** and then **[DONE]**.

## 7 Calculate Your Results

1. The SafTest™ analyzer will use the Calibrators to calculate the lipid peroxide content as milliequivalents of peroxides per kilogram of sample.
2. Adjust instrument results by taking into account the dilution factor. For example:

Dilution Factor	SafTest™ Results	Dilution x Results	Final Results
1:4	0.08 meq/kg	4 x 0.08 meq/kg	0.32 meq/kg

3. The final result can be calculated into milliequivalents of peroxides per kilogram of fat by taking the percent fat into consideration when performing your calculation. For example, with a sample that is 10% fat and yields a final result of 0.32 meq/kg:

Final Result	Result/(%Fat/100)	Final Result
0.32 meq/kg	0.32/(10/100)	3.2 meq/kg of fat

## 8 Storing Equipment

1. At the end of the day, store the Calibrators, Control, and reagent bottles (with dispensers attached) at 2–8°C.
2. To maximize lamp life, turn OFF the SafTest™ analyzer when not in use. Power OFF the SafTest™ analyzer by pressing the [O] marked portion of the power switch, which is located on the rear panel of the SafTest™ analyzer.

## Troubleshooting

If the sample value is greater than the value of the highest calibrator, the instrument will flag the results as “HI.” The sample must be prepared at a higher dilution and retested; see **Additional Dilutions QuickCard**.

If the sample value is less than the value of the lowest calibrator, the instrument will flag the results as “LO”. Values that are flagged “LO” should be reported as “< value of the lowest calibrator.”

Check the instrument printout for flags or error messages before reporting results. The coefficient of variation (%CV) should be less than 10%. Higher variations will be flagged, in which case you should repeat the test.

**EXCEPTION:** A large %CV is expected with samples that are measured at the low end of the calibration curve. For such measurements, do not repeat the test.

If the slope, intercept, or correlation coefficient guidelines are not met, recalibrate the instrument.

The range for the Control is found on the Peroxide Control Package Insert. Your test value for the Control should approximate this range. If it does not, rerun the test.

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