

Package Insert for *FASafe/AciSafe*™

FASafe/AciSafe™ Kit Package Insert & Control Package Insert

Introduction

FASafe™ standard test kit is designed to measure free fatty acid (FFA) content of oils and fats in fresh and used oils and in raw and finished products. Free fatty acids are a key indicator of hydrolytic degradation associated with off flavor and textural changes. The FFA is quantitated as percent oleic acid using an indicator that responds to the acids in the sample matrices. The practical dynamic range of the determination has been established as 0.3 to 1.8 percent oleic acid in these matrices. This test kit is not intended to accurately measure FFA levels lower than 0.3 percent. However, samples with higher levels can be evaluated and only require a dilution step prior to analysis. The Standard kit under validation overestimates the actual FFA concentrations below 0.3 percent. Consequently, sample matrices containing lower FFA levels must be tested using an alternative method (i.e., FFA High Sensitivity Kit).

Intended Use of Method

SāfTest, Inc.® has developed a proprietary test kit *FASafe*™ which is designed to provide rapid analysis of fat content in free fatty acid content of oils, tallows, greases, feed, and protein meals using micro-analytical and membrane separation principles.

The official American Oil Chemists' Society (AOCS) Ca 5a-40 method, commonly used in food testing laboratories, is based on titration of an ethanolic solution of the fat or oil material with ethanolic potassium hydroxide to a visually determined phenolphthalein endpoint. The conventional method requires large volumes of organic solvents, large sample aliquots, and it involves a lengthy titration process, as well as extraction for some samples which can take up to eighteen hours. The proprietary *FASafe*™ assay kit can be used for testing solubilized dry and wet food matrices by releasing lipid from the sample matrix using a stabilized reagent (i.e. stabilized isopropanol) and employing mechanical mixing and warming techniques. The solubilized food matrix is then filtered through a membrane and the filtrate is analyzed for free fatty acids. The significantly shorter sample preparation of 15 to 20 minutes permits data to be obtained in 15 to 25 minutes. The SAI's *FASafe*™ standard test kit can accommodate up to 135 test tubes and it contains all reagents, calibrators, and control standards required for testing. The test parameters for this procedure are presented below:

Sample Preparation:	<i>Liquid oils require no heating; semi-solid and solid oils and meals require heating</i>
Time Requirement:	<i>Sample Preparation 5 to 10 minutes</i>
	<i>Analysis Time approximately 5 to 10 minutes</i>
Limit of Quantitation:	<i>0.3%</i>
Interferences:	<i>Highly colored samples, samples containing acids, bases, or chelating metal ions</i>

Principle of Method

Samples are solubilized in the proprietary preparation reagent, separated through the membrane separation pack, and then analyzed by an optical reader in the presence of the chemical detectors. The test is based on the sensitivity of a chromagen to pH changes as a result of an increase in free fatty acids, which absorbs at 570nm. The increase in free fatty acids is measured as a decrease in the absorbance at 570nm. Lipid peroxide standards are assigned grams of oleic acid per 100 grams of sample. A reference wavelength at 690 nm is used.

Controls and Their Use

One control is supplied which can be analyzed as described in the control package insert and should fall within concentration ranges provided.

General Instructions

Test Kit Information

- a. **Kit Name:** Percent Fat Test Kit
- b. **Catalog Number:** 07PF1010
- c. **Ordering Information:** MP Biomedicals, LLC, www.mpbio.com/safest, 1.800.848.1163, safest@mpbio.com
- d. **Test Kit Reagents:**

Preparation Reagent and Reagent A – Isopropanol based reagent with the addition of stabilizers.

- **Reagent A** – Mixture of three enzymes to breakdown fat to glycerides and then glycerol for colorimetric quantitation. Reagent A is tested for presence of low degradants and low levels of antioxidants are added for stabilization.

Additional Supplies and Reagents

Membranes – High Non-Specific Adsorption membranes are used for prefiltration and consist of polypropylene or polyethersulfame or polythtrathoro ethylene structures.

Apparatus

Contents of Bench Top SafTest™ Platform include:

- a. **SafTest™** Analyzer
- b. **SafTest™** Filtration Unit
- c. Vortex
- d. Heating Block with tube Inserts
- e. Test Tube Racks (2)
- f. Positive Displacement Pipettes (2)
- g. Bottle Top Dispensers
- h. Multi Display Timer

Contents of Essential Disposable Labware:

- a. 15mL Conical Vials
- b. 10mm glass test tubes and caps
- c. Positive Displacement Pipette Tips
- d. Kim Wipes
- e. Glassbeads

Standard Reference Materials

Standard Solutions

Calibrators – One to five fixed concentrations of lipid peroxides prepared in stabilized isopropanol.

Controls –Mixture of triglycerides prepared in stabilized isopropanol.

Safety Precautions and Material Safety Data Sheets (MSDS)

All testing materials should be handled in accordance with good laboratory practices (GLP). Pipetting by mouth is highly discouraged. Eye protection that satisfies ANSI Z87.1, laboratory coat, and appropriate chemical resistant gloves must be worn while handling standards, reagents, and solvents. The Percent Fat reagent will stain clothing and equipment. Protective equipment that becomes contaminated must be removed and replaced as necessary. When used as directed, the Percent Fat reagents, controls, and calibrators should present no health hazard to the user. As a normal laboratory precaution, avoid contact with eyes and skin. The reagent is water based and requires no special disposal. The test does not have to be performed in a hood.

General Guidelines

To minimize contamination during the testing procedures and to maximize the quality of results, please perform the following:

1. Allow all calibrators, reagents, and dispensers to stand at room temperature (18 to 25°C) for 15 to 20 minutes prior to testing.
2. When transferring samples to the membrane units, use a new, disposable transfer pipette for each sample.
3. When using the positive displacement pipette:
 - a. Use a new tip for each sample, control, reagent blank, and calibrators.
 - b. Make sure the pipette tip is securely fastened to the pipette.
 - c. Eliminate all air bubbles from the pipette tip.
 - d. Gently wipe outside of the pipette tip before dispensing the sample to remove excess sample.
 - e. After dispensing the sample, make sure to touch the pipette tip to the inside of the assay tube to expel residual sample at the end of the tip.
4. When using the dispensers:
 - a. Read the dispenser instructions carefully before using.
 - b. Make sure the correct dispenser is placed on the appropriate reagent bottle.
 - c. Dispense five aliquots into a waste container to eliminate any air bubbles prior to each assay.
 - d. Slowly dispense reagents to prevent splashing.
 - e. Do not allow the tip of the dispenser to touch the sides of the tubes.
 - f. Use a new tip for each sample, control, reagent blank, and calibrators.
5. When using the SafTest™ Analyzer:
 - a. Refer to the SafTest™ Analyzer instructions detailed instructions and an explanation on error codes.
 - b. Allow the instrument to warm-up for at least 5 to 10 minutes.
 - c. Wipe tubes before analysis.
 - d. Make sure that calibration curve falls within specifications (See Section 13.3.5).
6. Store all reagents, controls, and calibrators in the refrigerator at approximately 2°C to 6 °C when not in use.
7. Discard reagents when all reagents in the kit have been exhausted.
8. Only fresh calibrators and Preparation Reagent must be used with new kits.
9. Properly discard any filtered samples, assay tubes, pipette tips, membrane holders, and membrane units.
10. *SafTest*™ kits must be refrigerated at 2° to 6°C in an upright position. **DO NOT FREEZE KITS.**
11. To maximize lamp life, turn off the *SafTest*™ Analyzer when not in use.

Sample Preparation

1. Allow the samples and reagents to reach at room temperature before testing.
2. Allow calibrators, Preparation Reagent, Controls, and PeroxySafe™ Reagent stand at room temperature (18-25°C) for 15-20 minutes.
3. Prepare the samples by following the procedures for the PeroxySafe™ assay.
4. For samples less than 20% expected % fat, prepare a 1:4 initial dilution as described on the sample preparation card. For samples with expected % fat of 20 to 40%, begin with an initial 1:8 dilution by weighing out 1.0 g and adding 7.0 mL of Prep Reagent. An even higher initial dilution may be required for highly saturated fats in products or higher fat products. Note: Keep solubilized samples warm when filtering and diluting to ensure the more saturated fats remain in solution.
5. For optimal results, the Control must fall within the acceptance range and the slope should be about 2.0 to 2.4. This provides the best range for quantitating percent fat in samples. Do not vortex tubes but invert 4x to mix. Please refer to the Control Insert for acceptance windows.
6. Samples should read above the first (lowest) calibrator and below the fourth calibrator to obtain accurate results
7. Turn heat block on low to 40°C (Dial number 4, $\pm 2^\circ\text{C}$). Check temperature.
8. Turn on the *SafTest*™ Analyzer and allow it to warm up.
9. Start the test using the PeroxySafe™ protocol. Set the timer and note the time the assay will be analyzed in the *SafTest*™ Analyzer.
10. Analyze the assay tubes in the *SafTest*™ Analyzer according to procedure given in Quick Start Cards.
11. Refer to Quick Start Cards for a detailed procedure of the analytical process.
12. For optimum results, a sample preparation and dilution scheme is provided in Table 2.

Table 2. Sample Preparation and Dilution for *SafTest* % Fat Test

Initial Preparation	Next Dilution	Final Dilution	Expected % Fat Range
1:4	None	1:4	0.0 - 1.0
1:4	1:2	1:8	1.0 - 2.0
1:4	1:4	1:16	2.0 - 4.0
1:4	1:8	1:32	4.0 - 6.0
1:4	1:12	1:48	6.0 - 10.0
1:4	1:32	1:128	10.0 - 20.0
1:8	1:25	1:200	20.0 - 30.0

1:8	1:50	1:400	30.0 - 60.0
1:8	1:60	1:480	60.0 - 100.0

13. To obtain good method precision, CV should be less than or equal to 10%. If CVs are higher, check sample or dilutions for homogeneity. Also, check to make sure that the results are in the optimal range of the calibration curve

Quality Control

- Calibration is required and calibrators are provided for this purpose.
- Calibration should always be performed with each new kit. For several tests within the same test kit, a STAT curve can be used recalling the last calibration curve. The slope must be linear with a correlation coefficient (r^2) at or above 0.990 or the instrument will stop and not read the sample responses.
- The control should be analyzed in each test run and should fall within the range stated on the control package insert.

Analytical Procedure

The SafTest, Inc. *PeroxySafe*[™] Standard Test Kit measures PV in samples using an electron transfer that responds to peroxides in the range of 0.01 to 0.5 expressed as meq of peroxide per kg of oil or fat. Samples with higher peroxide concentrations require additional dilutions before they can be analyzed by this method.

Dispenser and Reagent Preparation

- Allow reagents and dispensers to reach room temperature (i.e., 18 to 25 °C) before beginning assay.
- Mix the contents of the reagent bottles by swirling prior to attaching dispensers.
- Attach the dispenser labeled “PeroxySafe A” onto the reagent bottle labeled “PeroxySafe[™] Reagent A”. Dispenser volume is fixed; therefore, no adjustment is necessary.
- Attach the dispenser labeled “PeroxySafe B” onto the reagent bottle labeled “PeroxySafe[™] Reagent B.” Dispenser volume is fixed; therefore, no adjustment is necessary.
- Attach the dispenser labeled “*PeroxySafe*[™] C” on the reagent bottle labeled “*PeroxySafe*[™] Reagent C”. Dispenser volume is fixed; therefore, no adjustment is necessary.
- Attach the dispenser labeled “Preparation Reagent” onto the reagent bottle labeled “Preparation Reagent.” Adjust volume according to instructions provided in Quick Start Card.
- Dispense approximately 4 to 5 aliquots of each reagent into a waste container to eliminate any air bubbles in the dispensers prior to use. To prime dispensers, please read the instructions in the dispenser package.

Data Interpretation and Test Result Reporting

- The SafTest™ Analyzer will use the calibrators to calculate the triglyceride concentration in grams of triglyceride per 100 grams of sample.
- If the sample value is greater than the value of the highest calibrator, the instrument will flag the result as 'HI.' The sample must be diluted at a higher dilution and retested. Values that are flagged 'HI' are inaccurate and should not be reported.
- Check the instrument printout for flags or error messages before reporting results.
- For failed curve fit ($r^2 < 0.990$ or $r < 0.995$), repeat the assay.
- The range for the control is found on the package insert provided with the Percent Fat Control. The assay value for the control should approximate this range.
- Adjust instrument results by taking into account the dilution factor are tested by pipetting the samples initially as follows:

- **Example Calculations:**

Dilution Factor	SafTest™ Result (%)	Dilution x Result (%)	Corrected Final Result (%)	Final Result (%)
Weighed Sample (200X)	0.224	0.224 x200	44.8	44.8