"Touch Screen Easy"
Introducing the NEW SaTest II high sensitivity platform for food and biodiesel testing. The combination of touch screen based protocols with ready-to-use kits make your most demanding applications quick, simple and accurate. With R coefficient > 0.98, nanomolar sensitivity and low C.V. values, this AOAC Certified System and Kits are the most economical solution for accurate determination of Peroxide Value, Free Fatty Acids / Acid Number, Glycerin, Percent Fat and other analytes in your production, quality assurance and R&D environment.

www.mpbio.com/safetest
MP Biomedicals LLC, a global supplier of life science, diagnostics and quality control products, introduces a new platform for food and biodiesel analysis, the SafTest II. Touch-screen based protocols with ready-to-use kits makes determination of:

- Peroxide Value,
- Free Fatty Acids,
- Percent Fat,
- Glycerin and
- Other analytes quick, simple, accurate and safe.

The SafTest II platform is a complete turn-key system for all of your food and biodiesel analysis. Furthermore, the SafTest is the only analytical platform which uses non-toxic or unfriendly organic solvents in conjunction with its ability to perform a wide variety of food and biodiesel quality tests.

The SafTest Platform consists of:

- **SafTest II Analyzer** – A high performance, touch screen specialized photometer
- **SafTest Vacuum Filtration Unit** – A sample preparation system for quick and simple lipid fraction isolation without the pain of solid phase extraction and use of toxic and environmentally unfriendly solvents.
- **SafTest Heat Block and Vortex** – Heat block with different size test tube inserts and shaker for sample homogenization.
- **SafTest Dispensers** – Pre-set volumetric dispensers which allow for quick analysis
- **SafTest Kits** – Reagents and calibrators needed to quantify the specific analyte in questions

The Next Generation, SafTest II platform from the world’s leader in affordable food and biodiesel analysis is immediately available, to boost productivity of your lab. Find out why thousands of users worldwide already use SafTest platforms and kits, free of risk for you, with our 30 days money back /order cancellation guaranty.
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SafTest Platform

The SafTest® System is a rapid, standardized testing system to prepare and separate complex matrices for analyses. Currently available micro-analytical tests provide information on the fat quality and content of samples being tested. In other words, the SafTest® System provides a series of tests that can determine the freshness of oil components, even in the smallest amounts, in foods and cosmetics and other complex matrices.

A common concern with any laboratory test is: Do I need a full laboratory to perform this procedure? With the SafTest® System, the answer is no. In fact, the SafTest® System is designed to be extremely versatile and provide quick testing at every stage of production – from receiving to manufacturing to shipping. The SafTest® System, a small workstation, can be set up anywhere in your facility so that a designated tester can determine the freshness of products you may be receiving or your finished goods that you are about to ship out.

The SafTest® System can provide results in as short as 10 minutes and a complete panel of tests takes a maximum of two hours. Therefore, the SafTest® System should be run continuously throughout the day. Not only can a large number of samples be tested, but repeated testing can also provide an indication of product longevity and batch consistency. This rapid and easy collection of data will give the technician, the producer, or management a better understanding of the quality and consistency of product. The results obtained from the tests will provide an instantaneous picture of the overall freshness of a product. The sensitivity of the tests will provide data about product freshness that has never been available before.

The Equipment

Virtually anyone, in any industry can use the SafTest® System. The SafTest® System is designed for simplicity and speed.

There are eight different tests: four to detect oxidative degradants found in oils and fats and four to detect those same substances in more complex materials. What is a complex material? A complex material is any product that is not pure oil but has oil in it – basically anything and everything else. The main difference between the oil and fat kits and the complex material kits is the sensitivity of the tests themselves. The only differences between the four tests for both oil and complex material kits are the actual chemicals used for each test, the timing required to obtain results, and the settings that are used on the SafTest® Analyzer (see New SafTest Analyzer Section). By following the simple instructions presented in the SafTest® Binder and the Quick Start Cards, any individual within your organization can use the SafTest® System. With the help of this report, anyone will be able to understand the results.

Available Test Kits

The SafTest® System includes test kits which detect lipid peroxide values, malonaldehyde levels, alkenal levels, and free fatty acid levels as well as fat content. These items are degradants created in the process of oil oxidizing and becoming rancid. These compounds are formed as the oil or oil in a matrix degrades. In essence, the SafTest® System tests for freshness.

Quality Profiles

Once all of the SafTest® assays have been run, and all of the data for a product has been collected, the data can be transformed into a graphical representation that shows the overall condition of a product and its position along the path of oxidation towards rancidity. Once a user is familiar with what the SafTest® values mean for each test, the graphical representation that a Quality Profile provides gives the user a very thorough understanding of product freshness. Below is an example of two Quality Profiles of tuna (a filet, b ground) showing the impact of grinding.

1 Rancid: rancid means to have a foul odor of oxidized oils. This means that our conception of something that is rancid comes from our sensory abilities to detect rancidity. However, with new knowledge about the toxic effects of oxidized oils, it is becoming increasingly important to detect levels of oxidation that might not be apparent to an individual’s sense of smell. Also, the interconnected effects that different stages of oxidation have on the eventual rancidity of a product makes early detection vitally important in predicting shelf life of a substance.
NEW SafTestII Analyzer

Your EYE for Food and Biodiesel Assays

The SafTestII Analyzer is a simple, easy-to-use photometric analyzer designed specifically for SafTest’s food and biodiesel quality assays. The discrete, bi-chromatic photometer allows users to quickly analyze and calculate the amount of analytes present in food or biodiesel matrix in combination with the Safest Kits. Touch-screen based graphical user interface with pre-programmed protocols makes this instrument simple to learn and easy to operate. Menu-driven software provides flexibility for the user to perform a wide variety of assays in addition to adding more user specific protocols.

SafTestII Analyzer Key Features:

- Preprogrammed touch-screen based protocols for standard, complex matrix and high sensitivity determinations of:
  - Lipid peroxides (PV)
  - Free Fatty Acids
  - Percentage of Fat
  - Alkenals
  - Malonaledhyde
  - Total Glycerin
  - Free Glycerin
  - Bound Glycerin
  - Free Fatty Acids
  - Acid Number

- Easy custom programming for new assays.
- Reads standard disposable glass 10 or 12mm diameter round tubes, no expensive cuvettes necessary
- Results exported to both onboard printer or USB drive
- Large full color LCD touch screen (320x240 pixels) for easy navigation and viewing
- Linear and polygonal interpolation for test kit calibration
- Built in memory (64Mb) for saving and recalling previous calibration curves
- Onboard calculation of concentration or value indexes based on calibration curves
- Absorbance read time less than 2 seconds

SafTest II Specifications:

- Absorbance Range: 0.000-2.000 AU
- Wavelength: 550nm, 570nm and 690nm
- Optics: Bichromatic
- Reproducibility: R coefficient >.08; Standard deviation of < 0.005 AU when measured with 20 samples of distilled water
- Liquid Crystal Display: 320x240 STN LCD with Touch Screen
- Printer: Onboard printer
- Size: 30 cm x 27.2 cm x 12.5 cm of: 6.17 lbs (2.8 kg)
- Tube Size: 10 mm and 12 mm generic round-bottom glass or polystyrene
- Read Time: Less than 2 seconds per tube
Using the SafTestII Analyzer

Step 1: Analyzing Samples
- Samples should be prepared according to “Preparing Samples for STD Assays”.
- Label new 12mm glass test tubes per sample.
- Use a positive displacement pipette to transfer 200µl of each prepared sample into the labeled test tube.
- Dispense 1 aliquot of PeroxySafe Reagent A into every sample test tube.
- Dispense 1 aliquot of PeroxySafe Reagent B into every sample test tube.
- Dispense 1 aliquot of PeroxySafe Reagent C into every sample test tube.
- Once you have aliquoted Reagent C into the last test tube start the timer for 10 minutes.
- Cap the test tubes and vortex them at the fastest dial setting for 15 seconds.
- Place the test tubes back in the test tube rack for the remaining time.

Step 2: SafTest Analyzer Set Up
- Press [RUN] icon on the Touch Screen LCD

Step 3: Calculating Your Results
- The SafTest Analyzer will use the calibrators to calculate the lipid peroxide content as milli-equivalents of peroxides per kilogram of sample.

The SafTestII analyzer is an easy-to-use, WYSIWYG touch screen driven tool for the food and biodiesel industry. As shown in the figures above, the touch screen interface drives the test process in a logical step by step manner allowing users to quickly set up protocols and determine concentrations of analytes within various samples. Results are displayed on the color touch screen with the ability to print on an embedded printer or store as a *.CSV file via USB interface for easy data recall or documentation.
**SafTest Membrane Separation Unit**

**Rapid and Environmentally Friendly Isolation of Lipid Fractions**

For Solid Matrix Systems, the SafTest Membrane Filtration Unit is added to the platform to allow customers to rapidly isolate the lipid containing fraction without the use of long and toxic extractions. With the use of a variable vacuum, tube holder and specific filter paper, the Filtration Unit is able to quickly collect the lipid containing portion of ANY type of matrix. The collected fraction is then tested using the SafTest kits to determine fat content or lipid quality of the food matrix. The inexpensive and disposable filters eliminate the possibility for cross-contamination and allows up to 6 samples to be filtered at one time.

**Definition of SafTest Filtration Unit Parts:**
- **Vacuum Pump** - use vacuum of up to 20mmHg for rapid flow of homogenized matrices through the membrane
- **Acrylic Base** - 12mm test tube holder used to collect extracted filtrate
- **Disposable filter boats** for efficient and quantitative separation of liquid fraction:
  - Super-hydrophobic membrane, with 45 um pore size
  - Disposable membrane inserts, avoid cross-contaminate
  - 6 samples per disposable tray

**How the Safest Filtration Unit works?**

One gram of a sample is added to 3mL of SafTest's Proprietary Prep Reagent. The sample is then vortexed, mechanically disrupted and slightly heated for fifteen minutes to allow for complete extraction of fat into the SafTest Prep Reagent. To ensure proper isolation of the lipid containing fraction the slurry is then filtered through the Disposable Filter Boats using a vacuum as shown in the figure below. This collected filtrate can then be used with any of the SafTest STD, MSA or HSY kits for quantification of the specific analyte in question.
**Lipid Peroxide Analysis with PeroxySafe Kits**

**What are Lipid Peroxides?**

Lipid peroxides are the primary product of oxidized oils or fats. Virtually anything in this world can oxidize. What does oxidation mean? Oxidation can be thought of as “rusting”. When metal oxidizes we get rust, when fats and oils oxidize, we get lipid peroxides. Oxidation is the loss of electrons from atoms or molecules. When a molecule loses its electrons, it becomes a new and different type of molecule. Phospholipids, a typical lipid in animals and plants, is a major target for lipid oxidation due to its increased surface area and higher degree of unsaturation.

When lipids or phospholipids are exposed to air they react with Oxygen. Oxygen is very electro-negative, so it likes to grab positively charged toms or molecules like hydrogen, leaving other atoms or molecules with their electrons exposed. Compounds with two adjacent oxygens and no hydrogens or other positively charged atoms to couple to the exposed electrons are called peroxides, and are highlighted in the figure.

The peroxide formation accelerates formation of other peroxides in a propagation step. Peroxides are referred to as “free radicals” and there has been much emphasis in the press and from the health industry on preventing free radicals. “Free radicals” or peroxides will destroy the normal state of many chemicals in our bodies, in food products, and other biological matter. In food, the presence of peroxides will immediately alter the taste. Once oxidation has run its course, the oxidized material will have essentially changed to a new substance, chemically different from its original form and potentially toxic, which is why it is considered rancid and unusable.

**What is PeroxySafe?**

The PeroxySafe™ Kit is designed to provide rapid analysis of lipid peroxides in any type of oil, food or animal matrix using micro-analytical and membrane separation principles. The PeroxySafe™ quantitates peroxide value (PV) by transferring a free electron to a metal-chromogen complex whose visible spectrum then changes and is read using the SafTestII Analyzer. SafTest's PeroxySafe Kit was evaluated against the American Oil Chemists' Society (AOCS) Official Method Cd 8-53, commonly used in food testing laboratories. The AOCS 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 several hours. The proprietary PeroxySafe™ kit can be used for testing solubilized dry and wet food matrices by extracting lipid fat from the sample matrix using our Preparation Reagent and employing mechanical mixing and warming techniques. This results in significantly shorter sample preparation (15 minutes) allowing for data to be obtained in under 30 minutes. The PeroxySafe™ test kit contains all reagents, calibrators, and controls required for testing up to 110-120 tests. The PV value is expressed as milliequivalents of peroxide per kilogram of fat.

<table>
<thead>
<tr>
<th>Sample Preparation:</th>
<th>Liquid oils may require heating or dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Semi-solid/solid oils and meals require heating and mechanical disruption</td>
</tr>
<tr>
<td></td>
<td>Meals require membrane separation</td>
</tr>
<tr>
<td></td>
<td>Sample Preparation 5 to 20 minutes</td>
</tr>
<tr>
<td>Time Requirement:</td>
<td>Analysis time approximately 10 -15 minutes</td>
</tr>
<tr>
<td>Limit of Quantification:</td>
<td>0.02 to 50 meq/kg</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Kit</th>
<th>Catalog#</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Kit (STD)</td>
<td>07KTPR1020</td>
<td>Oils, hydrogenated oils, milk, eggs, nuts, grains, fried foods, crackers, chips, seeds</td>
</tr>
<tr>
<td>Matrix Special Kit (MSA)</td>
<td>07KTPR1010</td>
<td>Meals, ground meats, fish, pumpable meats and digestes, fresh organs, finished products (wet/dry), tallow and greases</td>
</tr>
<tr>
<td>High Sensitivity Kit (HSY)</td>
<td>07KTPR1030</td>
<td>Oils and hydrogenated oils</td>
</tr>
</tbody>
</table>
Lipid Peroxide Analysis with PeroxySafe Kit  
AOAC Certification# 030501

Summary of Validated Claims:
The PeroxySafe™ Kit is designed to measure milliequivalents (meq) of peroxide per kilogram (kg) of sample of oils and fats in fresh and used oils and in raw and finished products. The dynamic range of determination is 0.01 to 5.0 milliequivalents per kilogram (meq/kg) in the these matrices and four times these levels for solid matrix samples requiring solubilization and filtration prior to analysis. The SafTest Platform has demonstrated that the PeroxySafe™ Kit is capable of analyzing a wide-range of triglycerides with a high degree of accuracy and precision.

Sensitivity: Determination of Limit of Detection and Limit of Quantitation
For the PeroxySafe™, the LOD has been empirically determined as 3x SD of 10 replicate analyses of a low control and is calculated as 0.005 meq/kg PV. The empirical detection limit study has demonstrated that this method is capable of measuring peroxides down to 0.02-meq/kg levels with high degree of confidence.

Repeatability and Accuracy Studies
Strong agreement was observed based on correlation coefficient, r, of 0.99 and 0.97 achieved between the results generated using PeroxySafe™ and the traditional AOCS method on 40 meal samples and 276 different types of oil samples, respectively.

Lot-to-Lot Reagent Stability (Ruggedness) Study
In all cases, pooled RSD (CV) values were well below 10% indicating close agreement (precision) between each set of results generated using the PeroxySafe™. Variations observed between three lots of chemicals prepared at different times were insignificant.

Methodology
To demonstrate method repeatability, accuracy, and linearity, vegetable and marine (fish) oil samples, oil and seed oils, and meals and protein powders were sent to an independent method validation laboratory for validation. Samples were analyzed using the AOCS Official method Cd 8-53 and SafTest’s PeroxySafe Kit.

Vegetable oils, seed oils, marine oils, olive oils, pressed oils
Approximately 220 samples of various types of vegetable, seed, and marine oils were analyzed. Excellent agreement (i.e., correlation coefficient, r, 0.97 to 0.99) observed from method-to-method comparison of PeroxySafe™ and the traditional AOCS method for all oils.

Meals and protein powders
One hundred samples of 10 food categories of meals and protein powders were extracted and analyzed by the traditional AOCS method and using membrane separation by PeroxySafe. Excellent agreement (i.e., correlation coefficient, r, of 0.99) and linear relationship was observed between the two methods. This data validates the useful range of PeroxySafe™ from 0.2-10.0 meq PV/kg. Furthermore, the mean of 20 individual %RSDs (%CV) values yielded an estimate of the within-laboratory precision (repeatability) of 6% for meals.

Animal fats and oils
A strong correlation (i.e., correlation coefficient, r, of 0.99) and a linear relationship which were observed between the data generated using the PeroxySafe and the AOCS method for 100 samples of animal fats and oils. The mean of 20 individual %RSDs (%CV) yielded an estimate of the within-laboratory precision (repeatability) of 5 percent for animal fats and oils.
Free Fatty Acid Analysis with FaSafe/AciSafe Kits

What are Free Fatty Acids?
Fatty acids are the building blocks of fat sources in living organisms. Fat, or lipids are made up of 3 (fatty acids) attached to a glycerol backbone to make up a triglyceride. Since fatty acids are necessary to create essential building blocks such as triglycerides, they are rarely found floating alone within cells. When these acids are floating alone, they are referred to as free fatty acids. Free fatty acids appear as lipids breakdown products and are therefore good indicators of degradation. There are many types of free fatty acids. They can be differentiated by the length of the carbon chain, the presence and number of double bonds and the alignment of the carbons at the double bonds (cis- and trans- fatty acid). Free fatty acids have been shown to have both beneficial and adverse affects to health. Recently, trans-fatty acids (TFA) have even caught the attention of the FDA due to their negative health effects, and there is proposed new labeling on food packaging to indicate their presence. So, there is a great need in the food and cosmetic industries to have the ability to detect free fatty acids. Free fatty acids have a structure such as the diagram to the right (note that the “R” indicates an unspecified length chain of carbons).

What is FaSafe/AciSafe?
The FaSafe™ and AciSafe Kit are designed to provide rapid analysis of the free fatty acid content of oils, tallows, greases, feed, and protein meals using micro-analytical and membrane separation principles. The FaSafe™ and AciSafe quantitates free fatty acids (FFA) by using a pH sensitive chromogen whose visible spectrum then changes and is read using the SafTestII Analyzer. SafTest’s FaSafe™ and AciSafe Kit were evaluated against the American Oil Chemists’ Society (AOCS) Official Method Ca 5a-40 method, commonly used in food testing laboratories, which 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™ and AciSafe 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 25 to 30 minutes. MP Biomedicals FaSafe™ and AciSafe kit contains all reagents, calibrators, and controls required for testing up to 110-120 tests. The Free Fatty Acid value is expressed as percent oleic acid.

Sample Preparation: | Time Requirement: | Limit of Quantitation: | Interferences: |
--- | --- | --- | --- |
Liquid oils may require heating or dilution | Sample Preparation 5 to 20 minutes | 0.3% oleic acid | Highly colored samples, samples containing acids, bases, or chelating metal ions |
Semi-solid/solid oils and meals require heating and mechanical disruption | Analysis time approximately 10 minutes | | |
Meals require membrane separation | | | |

<table>
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<td>Matrix Special Kit (MSA)</td>
<td>07KTFA1010</td>
<td>Meals, ground meats, fish, pumpable meats and digests, fresh organs, finished products (wet/dry), tallow and greases</td>
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<tr>
<td>High Sensitivity Kit (HSY)</td>
<td>07KTFA1030</td>
<td>Oils, hydrogenated oils</td>
</tr>
</tbody>
</table>
Free Fatty Acid Analysis with FASafe™/AciSafe Kit

**Summary of Validated Claims:**
FASafe™/AciSafe test kit is designed to measure free fatty acid (FFA) content of fresh and used oils and fats 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 20% oleic acid. However, samples below 0.3% oleic acid must be tested using the High Sensitivity Kit.

**Sensitivity: Determination of Limit of Detection and Limit of Quantitation**
The LOD for the FASafe™/AciSafe has been empirically determined as 3x SD of 10 replicate analyses of a low control and is calculated as 0.05% oleic acid. The empirical detection limit study has demonstrated that this method is capable of measuring FFA down to 0.3% with 95% confidence.

**Repeatability and Accuracy Studies**
Strong agreement was observed based on the correlation coefficient, r, of 0.98 and 0.98 achieved between the results generated using FASafe™/AciSafe and the traditional AOCS method on 40 meal samples and 276 different types of oil samples, respectively.

**Lot-to-Lot Reagent Stability (Ruggedness) Study**
In all cases, pooled RSD (CV) values were well below 10% indicating close agreement (precision) between each set of results generated using the FASafe™/AciSafe kit. Variations observed between three lots of chemicals prepared at different times were insignificant.

**Methodology**
To demonstrate method repeatability, accuracy, and linearity, vegetable and marine (fish) oil samples, oil and seed oils, and meals and protein powders were sent to an independent method validation laboratory for validation. Samples were analyzed using the AOCS Official method Ca 5a-40 and SafTest's FASafe™/AciSafe Kit.

**Vegetable oils, seed oils, marine oils, olive oils, pressed oils**
Approximately 120 samples of various types of vegetable, seed, and marine oils were analyzed. Close agreement (i.e., correlation coefficient, r, 0.98 to 0.93) observed from method-to-method comparison of FASafe™/AciSafe and the AOCS method for all oils.

**Meals and protein powders**
One hundred samples of 10 food categories of meals and protein powders were extracted and analyzed by the traditional AOCS method and using membrane separation by FASafe™/AciSafe. Excellent agreement (i.e., correlation coefficient, r, of 0.99) and linear relationship was observed between the two methods. This data validates the useful range of FASafe™/AciSafe from 0 to 23%. In addition, the mean of 20 individual RSD (CV) values yielded an estimate of the within-laboratory precision (repeatability) of 2.2 percent for meals.

**Animal fats and oils**
Excellent agreement (i.e., correlation coefficient, r, of 0.99) was observed between the data generated using the FASafe™/AciSafe and the traditional AOCS method for 100 samples of animal fats and oils. Free fatty acid levels ranged from 1.0% to 13.0%. In addition, the mean of 20 individual RSDs (CV) values yielded an estimate of the within-laboratory precision (repeatability) of 2.5 percent for animal fats and oils.
**Percent Fat Analysis with Percent Fat Kit**

**AOAC certification #020501**

**Why Percent Fat Kit?**

There are nearly 30 conventional AOAC methods designed to measure fat content, which vary widely depending on type of food matrix. These methods include the butt-tube, Soxhlets, Mojonnier and Babcock (dairy products) and acid-hydrolysis. These methods, depending on conditions of digestion and extraction processes employed, determine triglyceride as well as other lipid components, which yield biased high results. Furthermore, conventional methods typically require the use of large volumes of toxic and flammable solvents, large sample aliquots, and extraction procedures, taking up to 18 hours.

The proprietary % Fat™ is an easy-to-use method capable of measuring fat content ranging from 0.01 to 99.6%. The method has been designed specifically to measure and quantitate “total triglycerides.” This approach is consistent with the Food and Drug Administration’s (FDA) Nutrition Labeling and Education Act (NLEA) where fat is defined as total lipid fatty acids expressed as “triglycerides.” The Percent Fat™ assay kit is designed for testing solubilized dry and wet food matrices by extracting lipid fat using a stabilized reagent (i.e. stabilized isopropanol) and employing mechanical mixing and warming techniques. The within-laboratory reproducibility (precision) ranges from 1 to 4%. The percent fat, measured as triglyceride concentration, is quantitated in grams (g) per 100 g of sample or % fat.

### Methodology

To demonstrate method repeatability, accuracy, and linearity, 56 samples of various types of meals and 48 samples of snack products were sent to an independent method validation laboratory for validation. Samples were analyzed using the AOCS Official method AOCS Aa 4-38 and SafTest’s Percent Fat Kit.

**Repeatability and Accuracy Studies**

Average percent recoveries and variability (%CV) were evaluated and determined demonstrating high level of measurement accuracy (102%) and precision (1.2%). Further evaluation was performed on spike recovery studies as described below:

- **Powders and Flakes** – Fresh egg white with a 0.003% fat and spiked with neutral triglyceride of 0.034% (Triolein). Recovery of the spike was 108%. Old egg white material used in second experiment with a recovery of the spike of 100%.
- **Snacks** – Crackers having 9.65% fat were spiked with 1.84% neutral triglyceride (Triolein). Recovery of the spike was 104%. Pretzels were also used on a spike recovery study which yielded spike at 99.8%.
- **Meals** – Chicken meal having a fat level of 1.22% was spiked with 1.42% neutral triglyceride (Triolein). The spike was recovered at 102%. A second chicken meal sample was also tested and the spike was recovered at 100%.

**Meals and Protein Powders, Dairy**

Fifty-six samples of various types of meals were analyzed using the SafTest Percent Fat test and the traditional AOCS method. The overall within-laboratory precision (repeatability) was 2.6 percent for Percent Fat method and 9.7 percent for AOCS method for meal samples. AOCS method also demonstrated higher within-laboratory variation (S.D. of 7%) in comparison to Percent Fat Test Kit (2.6%). Good correlation (r of 0.86 and 1, respectively) was observed between the methods for meals and snacks, respectively.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean % Fat</th>
<th>Variance S.D.</th>
<th>SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCS</td>
<td>13.9338</td>
<td>2.8197</td>
<td>0.3802</td>
<td>55</td>
</tr>
<tr>
<td>SafTest Percent Fat</td>
<td>12.4821</td>
<td>2.6742</td>
<td>0.3574</td>
<td>56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean % Fat</th>
<th>Variance S.D.</th>
<th>SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCS</td>
<td>8.0327</td>
<td>5.2526</td>
<td>0.7582</td>
<td>48</td>
</tr>
<tr>
<td>SafTest Percent Fat</td>
<td>10.1134</td>
<td>6.7392</td>
<td>0.9830</td>
<td>47</td>
</tr>
</tbody>
</table>

**Nuts, Nut Butter & Nut Flours**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean % Fat</th>
<th>Variance S.D.</th>
<th>SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCS</td>
<td>45.1611</td>
<td>15.3221</td>
<td>3.6115</td>
<td>18</td>
</tr>
<tr>
<td>SafTest Percent Fat</td>
<td>51.8912</td>
<td>14.7384</td>
<td>2.5656</td>
<td>33</td>
</tr>
</tbody>
</table>

**Dairy**

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean % Fat</th>
<th>Variance S.D.</th>
<th>SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOCS</td>
<td>38.4267</td>
<td>8.8686</td>
<td>2.0903</td>
<td>18</td>
</tr>
<tr>
<td>SafTest Percent Fat</td>
<td>38.4088</td>
<td>8.0311</td>
<td>1.3980</td>
<td>33</td>
</tr>
</tbody>
</table>
Glycerin Analysis with Total Glycerin Kit

Why Total Glycerin Kit?
Glycerol is an organic compound, also commonly called glycerin or glycerine. It is a colorless, odorless, viscous liquid that is large byproduct of the biodiesel industry. A by-product of the transesterification process is the production of glycerol. For every 1 tonne of biodiesel that is manufactured, 100 kg of glycerol are produced. The presence of glycerin in biodiesel, whether free or in combined form as mono-, di-, or triacylglycerols, indicates its incomplete removal in the generation of the fuel. Residual glycerin does not burn well and can lead to injector coking, reduced storage, and filter plugging due to sediment formation.

Sample Preparation:
- Biodiesel blends and B100 samples diluted 1:10 with preparation reagent
- Sample Preparation less then 5 minutes
- Analysis time 10 minutes
- 0.004% - 99.6% glycerin
- Samples containing glycerol and glycerol derivatives

Methodology
To demonstrate method repeatability, accuracy, and linearity, over 30 samples were sent to the NREL and 5 Lab currently using the SafTest Biodiesel Platform. Samples were analyzed using ASTMCS Official method AOCS Aa 4-38 and SafTest's Percent Fat Kit.

Repeatability and Accuracy Studies

![Graph showing comparison of ASTM Total Glycerin vs SafTest Total Glycerin]

![Graph showing comparison of Saftest Total Glycerin vs ASTM Total Glycerin]

![Graph showing comparison of various samples]

![Chemical structures of glycerol derivatives]
Malonaldehyde Analysis with AldeSafe Kit

What is Malonaldehyde?
Lipid peroxides and their hydrogen stripping powers will eventually degrade into other smaller products. One of the new products formed by these free radicals is malonaldehyde; and levels of malonaldehyde will increase in rancid foods, making it an excellent indicator of rancidity since these molecules have off odors and flavors. While scientific evidence has yet to find a direct link between malonaldehyde and cancer, it is considered a potential carcinogen. The basic structure of malonaldehyde is presented below. Note how it contains the three-carbon backbone like a triglyceride. One can imagine how the breakdown of a triglyceride can result in a malonaldehyde.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Catalog #</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Kit (STD)</td>
<td>07KTAD1020</td>
<td>Oils, hydrogenated oils, milk, eggs, nuts, grains, fried foods, crackers, chips, seeds</td>
</tr>
<tr>
<td>Matrix Special Kit (MSA)</td>
<td>07KTAD1010</td>
<td>Meals, ground meats, fish, digests, organs, finished products (wet/dry), tallow and greases</td>
</tr>
</tbody>
</table>

What is AldeSafe Kit?
The AldeSafe Kits are designed to provide rapid analysis of the malonaldehyde content of oils, tallows, greases, feed, and protein meals using micro-analytical and membrane separation principles. The AldeSafe Kit quantitates malonaldehyde (MDA) by through a condensation reaction where 2 MDA molecules react with a chromagen whose visible spectrum then changes and is read using the SafTestII Analyzer. The proprietary AldeSafe 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 MDA. The significantly shorter sample preparation of 15 to 20 minutes permits data to be obtained in 25 to 30 minutes. MP Biomedicals AldeSafe kit contains all reagents, calibrators, and controls required for testing up to 110-120 tests. The MDA value is expressed as µmol/kg.

Sample Preparation:
- Liquid oils may require heating or dilution
- Semi-solid/solid oils and meals require heating and mechanical disruption
- Meals require membrane separation

Time Requirement:
- Sample Preparation 5 to 20 minutes
- Analysis time approximately 20 minutes

Limit of Quantitation:
- 0-60mg/kg or 0-1,200µmol/kg MDA

Interferences:
- Highly colored samples

Methodology
To demonstrate method repeatability, accuracy, and linearity, samples of various types of meals were analyzed using the SafTest’s AldeSafe Kit.

Repeatability and Accuracy Studies

Quality Profile on Meat and Bone Meal Samples

Quality Profile on Fish Meal

SafTest® Results for Pet Food Samples Stored at 50°C for 52 to 194 days
Alkenal Analysis with AlkalSafe Kit

What are Alkenals?
Other products to arise from the degradation of lipid peroxides are alkenals. Like malonaldehyde, alkenals are products of secondary oxidation. As products of secondary oxidation, the presence of alkenals also is indicative of nanomoles with off odor and off flavors rancidity. There is evidence that the presence of certain alkenals within the body can affect cellular respiration and the presence of specific alkenals has been linked to such diseases as Alzheimer's. Shown below is one basic structure of an alkenal.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Catalog #</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Kit (STD)</td>
<td>07KTAD1020</td>
<td>Oils, hydrogenated oils, milk, eggs, nuts, grains, fried foods, crackers, chips, seeds. Meals, ground meats, fish, digests, organs, finished products (wet/dry), tallow and greases</td>
</tr>
</tbody>
</table>

What is AlkalSafe Kit?
The AlkalSafe Kit is designed to provide rapid analysis of the alkenal content of oils, tallows, greases, feed, and protein meals using micro-analytical and membrane separation principles. The AlkalSafe Kit quantitates alkenals through a condensation reaction with a chromagen whose visible spectrum then changes and is read using the SafTestII Analyzer. The proprietary AldeSafe 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 alkenals. The significantly shorter sample preparation of 15 to 20 minutes permits data to be obtained in 25 to 30 minutes. MP Biomedicals AlkalSafe kit contains all reagents, calibrators, and controls required for testing up to 110-120 tests. The MDA value is

Methodology
To demonstrate method repeatability, accuracy, and linearity, samples of various types of meals and samples of snack products were sent to an independent method validation laboratory for validation. Samples were analyzed using the AOCS Official method AOCS ρ-Anisidine / Gas Chromatography of Hexanal Content and SafTest’s AlkalSafe Kit.

Repeatability and Accuracy Studies

Correlation of AlkalSafe™ Results and Sensory Results

Correlation of AlkalSafe™ Results and p-Anisidine for Oil Samples

AlkalSafe™ - A Better Indicator of Oil Abuse than p-Anisidine