



# CERTIFICATION

**AOAC Research Institute**  
***Performance Tested Methods*<sup>SM</sup>**

Certificate No.

**052004**

The AOAC Research Institute hereby certifies the method known as

**SafTest Free Fatty Acid Test**

manufactured by

**MP Biomedicals**

**29525 Fountain Parkway**

**Solon, Ohio USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

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Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date

December 12, 2025

Expiration Date

December 31, 2026

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<b>METHOD NAME</b> SafTest Free Fatty Acid Test	<b>CATALOG NUMBER</b> 07KTFA2000
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<b>INDEPENDENT LABORATORY</b> Merieux NutriSciences Siliker Food Science Center (FSC) 111 East Wacker Dr. Chicago, IL 60601 USA
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<b>APPLICABILITY OF METHOD</b> Analyte – Free fatty acid content as percent oleic acid equivalent.  Matrixes – vegetable oils (1:1); fish oil (1:1); animal fats (tallows) (1:10); meat meal and fish meal products (1:4); potato chips, crackers, and other grain-based products (1:4).	<b>REFERENCE METHOD</b>  Official Methods and Recommended Practices of the AOCS (1996) 11 <sup>th</sup> ed. AOCS, Method AOCS Ca 5a-40, revised 2017. (2)
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Performance claims – The practical dynamic range of the calibration is 0.04% to 2.06% free fatty acids. For materials analyzed neat (e.g., vegetable oils, refined oils), the limit of quantitation (LOQ) is 0.05 % free fatty acids. For materials that are diluted 1:4 prior to testing (e.g., meat meals, snacks), the LOQ is 0.16% free fatty acids. For materials that are diluted 1: 10 prior to testing (e.g., fats, tallows), the LOQ is 0.40% free fatty acids. Materials with free fatty acid levels above the determination range can be analyzed by including a dilution step prior to analysis. The relative standard deviations of repeatability (RSD<sub>r</sub>) ranged from 1.1% to 8.1 % across all method developer matrix studies. Similar results were observed in the independent laboratory study where RSD<sub>r</sub> was found to be in the range 2.6-13%. Free fatty acid results by this method are in good agreement with results by AOCS Ca 5a-40 Method.

<b>ORIGINAL CERTIFICATION DATE</b> May 15, 2020	<b>CERTIFICATION RENEWAL RECORD</b> Renewed through December 2026.
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<b>METHOD MODIFICATION RECORD</b> NONE	<b>SUMMARY OF MODIFICATION</b> NONE
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Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 052004 this method is distributed by: NONE	Under this AOAC <i>Performance Tested Methods</i> <sup>SM</sup> License Number, 052004 this method is distributed as: NONE
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**PRINCIPLE OF THE METHOD (1)**

The SafTest System, a series of innovative test kits used in determining lipid quality and food freshness was developed to address these challenges. The SafTest System is a series of micro-analytical techniques based on membrane separation technology as an alternative to sensory and traditional analytical methods. One test of the series measures free fatty acids which are a key indicator of hydrolytic degradation associated with off flavor and textural changes. Results are standardized, objective, and rapid, with strong correlations to published, official test methods. Standardized, well documented, and user-friendly procedures improve productivity, performance, and quality control.

One of the methods in the SafTest System is the Free Fatty Acid Test. Food materials are solubilized in the preparation reagent, separated through the membrane separation pack, and then analyzed by an optical reader.

Liquid test portions are solubilized in the proprietary preparation reagent and then tested. For dry materials, test portions are solubilized and then separated through the membrane separation pack, tested and then analyzed by an optical reader. The test is based on a pH indicator reaction that takes place in a stabilized reagent. Free fatty acid calibrant concentrations are based on a % oleic equivalent. The decrease in indicator absorbance is proportional to the concentration of free fatty acids available to interact with the indicator. The indicator visible spectrum shifts with decreasing pH to absorption maximum in the 400 to 500 nm range. The resulting absorbance values are logarithmically related to free fatty acid concentration and decrease with increasing free fatty acid concentration.

#### DISCUSSION OF THE VALIDATION STUDY (1)

For materials analyzed neat (e.g., vegetable oils, refined oils), the LOQ is 0.05% free fatty acids. For materials that are diluted 1:4 prior to testing (e.g., meat meals, snacks), the LOQ is 0.16% free fatty acids. For materials that are diluted 1: 10 prior to testing (e.g., fats, tallows), the LOQ is 0.40% free fatty acids.

The method was demonstrated to be selective for twenty short and long chain fatty acids. All but five of 22 potential interferents produced responses below the LOQ, with two providing notable interference (1,6-hexanediamine and lauryl sulfate lithium).

This validation study yielded good agreement between SafTest Free Fatty Acid Test and the traditional AOCS Ca 5a-40. Mean recoveries from replicate analyses of free fatty acid solutions prepared at three different concentrations yielded recoveries ranging from 97 to 106 percent, well within the AOAC-recommended accuracy level of 80 to 120 percent. The relative standard deviations of repeatability (RSD<sub>r</sub>) for the SafTest Free Fatty Acid Test ranged from 1.1% to 8.1 % across all method developer matrix studies and were very similar to the RSD<sub>r</sub> obtained using AOCS Ca 5a-40 which ranged from 0.3% to 8.7%. The biases between the SafTest Free Fatty Acid Test and AOCS Ca 5a-40, expressed as a percent recovery from AOCS Ca 5a-40, ranged as follows: for olive oils, from 79% to 108%, and averaged 96.5%; for animal fats, from 80% to 102%, and averaged 94.0%; for meat meals, ranged from 92% to 109%, and averaged 103.9% (excluding the problematic poultry meal).

Independent laboratory study performed at Merieux NutriSciences confirmed method developer studies. Similar results were observed in the independent laboratory study where RSD<sub>r</sub> of the SafTest Free Fatty Acid Test method was found to be in the range 2.6-13%. Biases as reported in the independent laboratory study across all matrixes, expressed as a percent recovery from AOCS Ca 5a-40, ranged from 91.8% to 114.4% (with removal of one chicken meal outlier), and averaged 103.8%. Spike recovery of free fatty acid from three meat meals was found to be satisfactory ranging from 89-101.6%, with RSD<sub>r</sub> ranging from 2.65-8.12%.

The MP Biomedicals SafTest Free Fatty Acid Test Kit proves to be an acceptable alternative method to the traditional AOCS Ca 5a-40 test and very valuable for food matrixes where sufficient fat for reference method testing cannot be obtained. Using small reagent volumes, instrumental analysis and rapid detection times, and easy-to-use, standardized procedures, the SafTest Free Fatty Acid Test can determine free fatty acid levels in specific food matrixes with high degree of accuracy and precision.

During these comprehensive validation studies, several noteworthy observations were made.

**(a)** Inorganic acids in food matrixes can cross react with the SafTest Free Fatty Acid Test method.

**(b)** Intensely colored oils interfere with the AOCS Ca 5a-40; however, the dilution of these dark oils in the SafTest Free Fatty Acid Test method eliminates any color interference.

**(c)** A limitation of this test kit is that it is not suitable for measurements of free fatty acid in refined oils below the matrix LOQ of 0.05% and in snack products below the matrix LOQ of 0.16%. There are some applications when a lower concentration would be of interest.

**(d)** In the Independent Laboratory Validation, the end point detection using AOCS Ca 5a-40 was problematic when extracted fat was colored and the titration endpoint could not be observed. For meat meals where AOCS Ca 5a-40 was not dependable, spike recovery was used to evaluate accuracy of the SafTest Free Fatty Acid Test. Recoveries ranged from 89 to 102% for meat and fish meals, and repeatability ranged from 4 to 8%, both of which meet acceptance criteria.

Based on these experiences, meat meals, particularly meat and bone meal, should be removed from the scope of AOCS Ca 5a-40.

**Table 9. Method Developer Matrix Study on Olive Oils (1)**

Matrix	Method	n	Free Fatty Acid, % <sup>a</sup>	s <sub>r</sub>	RSD, %	Bias <sup>b</sup>
Olive Oil A	AOCS Ca 5a-40	10	0.4298	0.0104	2.4	-0.014
	SafTest FFA	10	0.4159	0.0151	3.6	
Olive Oil B	AOCS Ca 5a-40	10	0.2155	0.0028	1.3	-.004
	SafTest FFA	9 <sup>c</sup>	0.2115	0.0100	4.7	
Olive Oil C	AOCS Ca 5a-40	10	0.3349	0.0058	1.7	0.051
	SafTest FFA	10	0.3490	0.0110	3.2	
Olive Oil D	AOCS Ca 5a-40	10	1.2466	0.0204	1.6	-.1222
	SafTest FFA	10	1.1284	0.0161	1.4	
Olive Oil E	AOCS Ca 5a-40	10	0.6762	0.0144	2.1	-0.034
	SafTest FFA	10	0.6323	0.0281	4.4	
Olive Oil F	AOCS Ca 5a-40	10	1.2401	0.0188	1.5	-0.339
	SafTest FFA	10	0.9213	0.0754	8.1	
Olive Oil G	AOCS Ca 5a-40	10	0.9550	0.0159	1.7	-0.095
	SafTest FFA	10	0.8599	0.0448	5.2	
Olive Oil H	AOCS Ca 5a-40	10	1.3073	0.0083	0.6	-0.195
	SafTest FFA	10	1.1123	0.0330	2.9	
Olive Oil I	AOCS Ca 5a-40	10	0.2363	0.0034	1.4	0.002
	SafTest FFA	10	0.2382	0.0092	3.9	
Olive Oil J	AOCS Ca 5a-40	10	0.3007	0.0051	1.7	0.100
	SafTest FFA	10	0.3107	0.0101	3.3	
Olive Oil K	AOCS Ca 5a-40	10	0.2306	0.0029	1.3	0.180
	SafTest FFA	10	0.2486	0.0087	3.5	
Olive Oil L	AOCS Ca 5a-40	10	0.3920	0.1094	28 <sup>d</sup>	0.208
	SafTest FFA	10	0.1885	0.0106	5.6	

<sup>a</sup> Mean Fatty Acid Test, %<sup>b</sup> Bias calculated as SafTest Free Fatty Acid Test result minus AOCS Ca 5a-40 result<sup>c</sup> container broke and test was lost<sup>d</sup>olive oil was very dark and difficult to titrate

**Table 10: Method Developer Matrix Study on Meat Meals and Fish Meal (1)**

Sample Matrix	Method	n	Free Fatty Acid, % <sup>a</sup>	s <sub>r</sub>	RSD, %	Bias <sup>b</sup>
Lamb Meal #5	AOCS Ca 5a-40	10	17.649	0.354	2.0	1.355
	SafTest FFA	10	19.054	0.274	1.4	
Poultry Meal	AOCS Ca 5a-40	10	10.234	0.105	1.0	2.579
	SafTest FFA	10	12.763	0.245	1.9	
Fish Meal #2	AOCS Ca 5a-40	10	19.417	0.290	1.5	-0.577
	SafTest FFA	10	18.840	0.237	1.23	
A Poultry Meal #21	AOCS Ca 5a-40	10	11.622	0.109	0.9	0.598
	SafTest FFA	10	12.210	0.139	1.1	
Fish Meal #1	AOCS Ca 5a-40	10	23.115	0.437	1.9	0.939
	SafTest FFA	10	24.054	0.262	1.1	
Lamb Meal #12	AOCS Ca 5a-40	10	2.904	0.082	2.8	0.996
	SafTest FFA	10	3.900	0.190	4.9	
Meal #13	AOCS Ca 5a-40	9 <sup>c</sup>	18.488	0.471	2.5	1.026
	SafTest FFA	10	19.514	0.436	2.2	
Meat Bone Meal	AOCS Ca 5a-40	10	3.353	0.282	8.4	0.390
	SafTest FFA	10	3.740	0.158	4.2	
Poultry Meal #4	AOCS Ca 5a-40	10	11.22	0.036	0.3	0.040
	SafTest FFA	10	11.26	0.272	2.4	
Poultry Meal #35	AOCS Ca 5a-40	10	12.84	0.085	0.7	0.40
	SafTest FFA	10	13.24	0.281	2.1	

<sup>a</sup> Mean Fatty Acid Test, %<sup>b</sup> Bias calculated as SafTest Free Fatty Acid Test result minus AOCS Ca 5a-40 result<sup>c</sup> insufficient fat for titration in test portion

Table 11. Method Developer Matrix Study on Animal Fats and Oils (1)

Matrix	Method	N	Free Fatty Acid,% <sup>a</sup>	s <sub>r</sub>	RSD, %	Bias <sup>b</sup>
Animal Fat #1	AOCS Ca 5a-40	10	1.160	0.023	1.9	-0.085
	SafTest FFA	10	1.075	0.025	2.3	
Poultry Fat #1	AOCS Ca 5a-40	10	1.031	0.008	0.8	-0.046
	SafTest FFA	10	0.985	0.025	2.5	
Poultry Fat #3	AOCS Ca 5a-40	10	1.897	0.008	0.4	-0.049
	SafTest FFA	10	1.848	0.056	3.0	
Turkey Fat #1	AOCS Ca 5a-40	10	1.202	0.104	8.7	-0.066
	SafTest FFA	10	0.936	0.049	5.2	
Turkey Fat B	AOCS Ca 5a-40	10	1.031	0.008	0.8	-0.046
	SafTest FFA	10	0.985	0.025	2.5	
Poultry Fat #10	AOCS Ca 5a-40	10	1.840	0.029	1.6	-0.060
	SafTest FFA	10	1.900	0.078	4.1	
Animal Fat #19	AOCS Ca 5a-40	10	1.765	0.022	1.2	-0.035
	SafTest FFA	10	1.733	0.066	3.8	
Poultry Fat from Meal #35	AOCS Ca 5a-40	10	12.844	0.085	0.7	-0.036
	SafTest FFA	10	12.483	0.453	3.6	
Poultry Fat from Meal #4	AOCS Ca 5a-40	10	11.222	0.036	0.3	-1.702
	SafTest FFA	10	9.528	0.217	2.3	
Turkey Fat #7	AOCS Ca 5a-40	10	0.940	0.006	0.6	0.019
	SafTest FFA	10	0.959	0.034	3.5	

<sup>a</sup> Mean Fatty Acid Test, %

Table 12. Independent Laboratory Matrix Study (1)

Matrix	n	Parameter	Free Fatty Acid, %																																																																																																																															
			SafTest FFA (as is basis)	SafTest FFA (fat basis) <sup>a</sup>	AOCS Ca 5a-40 (as is basis) <sup>b</sup>	AOCS Ca 5a-40 (fat basis) <sup>a</sup>	Recovery, % <sup>c</sup> (as is basis)	Recovery, % <sup>d</sup> (fat basis)																																																																																																																										
Olive Oil	8	Mean	0.308	0.308	0.313	0.313	98.5 (p=0.789)	98.5																																																																																																																										
		%RSD <sub>r</sub>	13.3	13.3	9.0	9.0			Soybean Oil	8	Mean	<0.04	<0.04	0.090	0.090	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			20.6	20.6	Clarified Butter	8	Mean	0.236 <sup>d</sup>	0.236 <sup>d</sup>	0.258	0.258	91.8 <sup>f</sup> (p=0.012)	91.8 <sup>f</sup>	%RSD <sub>r</sub>	6.7	6.7	5.0	5.0	Beef Fat	8	Mean	0.593	0.593	0.519	0.519	114.4 <sup>f</sup> (p < 0.00)	114.4 <sup>f</sup>	%RSD <sub>r</sub>	6.1	6.1	6.6	6.6	Crackers	8	Mean	<0.16	<0.58	0.069	0.235	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			16.4	10.9	Baked Corn Scoops	8	Mean	<0.16	<2.00	0.035	0.443	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			15.3	9.5	Potato Chips	8	Mean	<0.16	<0.43	0.064	0.170	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			16.6	6.3	Chicken Meal	8	Mean	1.28 <sup>d</sup>	9.28 <sup>d</sup>	0.401	2.91	320.2 <sup>f</sup> (p < 0.001)	318.7 <sup>f</sup>	%RSD <sub>r</sub>	2.6	2.6	5.9	3.9	Meat & Bone Meal	8	Mean	2.21	22.45	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	%RSD <sub>r</sub>	5.5	5.5			Fish Meal	8	Mean	2.17 <sup>d</sup>	19.36 <sup>d</sup>	1.963 <sup>d</sup>	17.503 <sup>d</sup>	110.5 <sup>d</sup> (p < 0.001)	110.6 <sup>d</sup>	%RSD <sub>r</sub>
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		%RSD <sub>r</sub>			16.4	10.9			Baked Corn Scoops	8	Mean	<0.16	<2.00	0.035	0.443	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			15.3	9.5	Potato Chips	8	Mean	<0.16	<0.43	0.064	0.170	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			16.6	6.3	Chicken Meal	8	Mean	1.28 <sup>d</sup>	9.28 <sup>d</sup>	0.401	2.91	320.2 <sup>f</sup> (p < 0.001)	318.7 <sup>f</sup>	%RSD <sub>r</sub>	2.6	2.6	5.9	3.9	Meat & Bone Meal	8	Mean	2.21	22.45	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	%RSD <sub>r</sub>	5.5	5.5			Fish Meal	8	Mean	2.17 <sup>d</sup>	19.36 <sup>d</sup>	1.963 <sup>d</sup>	17.503 <sup>d</sup>	110.5 <sup>d</sup> (p < 0.001)	110.6 <sup>d</sup>	%RSD <sub>r</sub>	5.2	5.2	5.8	5.5																																																				
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		%RSD <sub>r</sub>			15.3	9.5			Potato Chips	8	Mean	<0.16	<0.43	0.064	0.170	NC <sup>e</sup>	NC <sup>e</sup>	%RSD <sub>r</sub>			16.6	6.3	Chicken Meal	8	Mean	1.28 <sup>d</sup>	9.28 <sup>d</sup>	0.401	2.91	320.2 <sup>f</sup> (p < 0.001)	318.7 <sup>f</sup>	%RSD <sub>r</sub>	2.6	2.6	5.9	3.9	Meat & Bone Meal	8	Mean	2.21	22.45	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	NA <sup>g</sup>	%RSD <sub>r</sub>	5.5	5.5			Fish Meal	8	Mean	2.17 <sup>d</sup>	19.36 <sup>d</sup>	1.963 <sup>d</sup>	17.503 <sup>d</sup>	110.5 <sup>d</sup> (p < 0.001)	110.6 <sup>d</sup>	%RSD <sub>r</sub>	5.2	5.2	5.8	5.5																																																																		
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<sup>a</sup>Fat tested by respective reference method.

<sup>b</sup>AOCS = AOCS Method Ca 5a-40 (Reference Method)

<sup>c</sup>Recovery calculated as SafTest Free Fatty Acid Test (as is basis) result divided by AOCS Ca 5a-40 (as is basis) result x 100

<sup>d</sup>Recovery calculated as SafTest Free Fatty Acid Test (fat basis) result divided by AOCS Ca 5a-40 (fat basis) result x 100

<sup>e</sup>NC = Not calculated.

<sup>f</sup>Values are significantly different (p < 0.05).

<sup>g</sup>NA = Not available (could not extract enough fat).

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