CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

102001

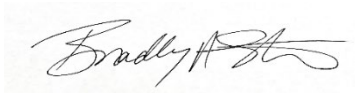
The AOAC Research Institute hereby certifies the method known as

SafTest Peroxide Test Kit

manufactured by

MP Biomedicals
29525 Fountain Parkway
Solon, Ohio 44139 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ 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*℠ 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.



Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date December 12, 2025

Expiration Date December 31, 2026

AUTHORS
Virginia C. Gordon, Christopher C. Rainey, and Willainia C. Studmire

SUBMITTING COMPANY
MP Biomedicals
29525 Fountain Parkway
Solon, Ohio 44139 USA

METHOD NAME
SafTest Peroxide Test Kit

CATALOG NUMBER
07KTPR2000

INDEPENDENT LABORATORY
Merieux NutriSciences
Silliker Food Science Center (FSC)
3600 Eagle Nest Dr.
Crete, IL 60417 USA

CONTROL LABORATORIES
Medallion Labs
4000 Plymouth Avenue
Minneapolis, MN 55427 USA

Pilot Laboratory
118 Veterinary Rd
Saskatoon, SK S7N2R4 Canada

APPLICABILITY OF METHOD

Target Analyte – Peroxide content as milliequivalents of peroxide per kg sample. Note: The test result can be converted to meq/kg fat using the fat content.

Matrixes – Vegetable oils (1:1; 200 µL), animal fats (tallows) (1:10; 0.2 g); meat meal and fish meal products (1:4; 1 g); and potato chips, and crackers and other processed grain-based snack products (1:4; 1 g).

Performance claims – The practical dynamic range of the calibration is 0.00 to 0.20 meq/kg. Materials with peroxide levels above this range can be analyzed by including a dilution step prior to analysis. For materials analyzed neat (e.g., vegetable oils, refined oils), the limit of quantitation (LOQ) is 0.02 meq/kg. For materials that are diluted 1:4 prior to testing, the LOQ is 0.08 meq/kg. If the matrices require preparation through the membrane and standard 1:4 dilution, the LOQs are higher (0.22 meq/kg for crackers and 0.32 meq/kg for chips). For materials that are further diluted to accommodate the peroxide concentration (e.g., final dilution ranging from 1:8 to 1:16), the LOQ ranges from 0.22 meq/kg peroxide to 0.46 meq/kg. The relative standard deviations of repeatability (RSD_r) ranged from 1-17 % across all method developer matrix studies in comparison of the AOCS Cd 8-53 (2) of 1-23% in these studies. Similar repeatability was observed in the independent laboratory study where RSD_r was found to be in the range of 2.4-11% for the SafTest Peroxide test and 5.4-12.4% for AOCS Cd 8b-90 (3).

REFERENCE METHODS

Official Methods and Recommended Practices of the AOCS (1997) 5th Ed., The American Oil Chemists' Society, Champaign, IL. Method Cd 8-53 (2) which is also referenced as AOAC Official Method 965.33. (3)

Official Methods and Recommended Practices of the AOCS (2017) 7th Ed., The American Oil Chemists' Society, Urbana, IL. Method Cd 8b-90. (4)

ORIGINAL CERTIFICATION DATE
October 15, 2020

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **December 2021 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes.**

Under this AOAC *Performance Tested Methods℠* License Number, 102001 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 102001 this method is distributed as:
NONE

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 challenges facing the food industry for more rapid, objective and environmentally friendly methods. 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 SafTest series is the Peroxide Test which measures peroxide values, key indicators of oxidative degradation and off flavor and rancidity. 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.

In this system, food matrices are solubilized in the preparation reagent, separated through the membrane separation pack, and the extracts are analyzed by an optical reader. The Peroxide Test quantitates peroxide value by transferring a free electron to a metal-chromogenic complex with visible spectrum increases with increasing peroxide concentration at 590 nm. Calibrators with known peroxide values in meq/kg are used to convert the optical density readings to peroxide concentrations.

DISCUSSION OF THE VALIDATION STUDY (1)

In the matrix studies, it was demonstrated that many elements such as the sample handling, preparation, timing of the tests, sample processing at higher temperatures for saturated samples, or incomplete fat separation are critical to obtaining accurate SafTest Peroxide Test results.

Given these limitations, the studies demonstrated the SafTest Peroxide Test demonstrated the accuracy and precision in spike recovery studies. Average recoveries in four matrix studies averaged 95.7 (olive oil), 91.5 (meat meals and fish meal), 105.2 (animal fats and oils) and 112 (independent laboratory validation). RSD_r % for the four matrix studies were very similar for the SafTest Peroxide Test and the AOCS methods ranging in general from 2-17%,

These studies demonstrated that the SafTest Peroxide Test proves to be an acceptable alternative method to the traditional AOCS Cd 8-53 or AOCS Cd 8b-90 Peroxide Value methods which require large volumes of highly toxic solvents, produces highly variable results, and are labor intensive procedures for food matrices. The SafTest Peroxide test uses small sample and reagent volumes, instrumental analysis and rapid detection times, and easy-to-use, standardized procedures. The SafTest Peroxide Test can detect varying peroxide levels in a variety of oils, fats, meat meals and fish meal, and snack products with good degree of accuracy and satisfactory precision. The SafTest is an acceptable alternative to AOCS Cd 8-53 and AOCS Cd 8b-90.

Problems encountered in the validation include: changes in peroxides concentrations with time; incomplete solubilization of the saturated fats; and the loss of fat during the grinding step due to over-grinding/heat generation.

- Materials with saturated fats must be prepared at 46-50°C for complete solubilization, complete fat recoveries and accurate peroxide results. In the independent laboratory validation study, high dilutions were used to compensate for use of preparation temperatures at 37–44°C for butter and beef tallow introducing unintentional error.
- High fat snacks must be very carefully ground to prevent incomplete fat recoveries and ultimately affect peroxide values. Over-grinding may account for lower recoveries of peroxide observed for potato chips in the independent laboratory validation.
- Critical to any peroxide comparability study is determining the peroxide values by both methods on the same day. In the method developer matrix study for snacks, the SafTest Peroxide Test and the AOCS reference method were performed five days apart. The peroxide values were changing rapidly with time, introducing an uncontrolled bias which prevented making direct method comparisons. Lesson learned: comparing peroxide results between methods requires that timing and handling be very carefully controlled.

The repeatability of the SafTest method was tighter than the reference methods in the Independent Laboratory Validation study, which is an important finding.

Table 9. Method Developer Matrix Study: Snack Samples. (1)

Matrix	Method	N[a]	Peroxide, meq/kg[b]	s_r	RSD_r, %[a]	Recovery, %[c]
Potato chip	AOCS Cd 8-53	8	0.53	0.01	1.9	
	SafTest Peroxide Test	8	2.09	0.32	15.0	394
Potato chip kettle	AOCS Cd 8-53	8	5.99	0.30	6.0	
	SafTest Peroxide Test	8	9.43	0.31	3.3	154
Honey grahams	AOCS Cd 8-53	8	0.47	0.05	10.6	
	SafTest Peroxide Test	8	1.54	0.13	8.6	328
Wheat cracker	AOCS Cd 8-53	8	8.23	0.10	1.2	
	SafTest Peroxide Test	8	9.13	0.46	5.0	111
Round cracker	AOCS Cd 8-53	8	1.56	0.16	10.2	
	SafTest Peroxide Test	8	4.92	0.08	1.0	321
Tortilla chips	AOCS Cd 8-53	8	1.56	0.16	10.2	
	SafTest Peroxide Test	8	2.30	0.21	9.1	147
Baked potato chips	AOCS Cd 8-53	8	1.37	0.18	13.1	
	SafTest Peroxide Test	8	2.66	0.22	8.9	194
Sweet crackers	AOCS Cd 8-53	8	0.56	0.04	7.1	
	SafTest Peroxide Test	8	2.31	0.54	23.0	412

[a] N = number of replicates

[b] Average test result, meq/kg, for respective method (8 replicates)

[c] Recovery of peroxide by SafTest Peroxide Test, as a % of AOCS Cd 8-53. Due to unavoidable circumstances SafTest Peroxide Tests were made 5 days later than AOCS Cd 8-53 and peroxide concentrations had increased dramatically.

Table 10: Matrix Study: Olive Oils (1)

Matrix	Method	N[a]	Mean Peroxide, meq/kg	s_r	RSD_r, %	t value	Recovery, %[b]
Olive Oil 1	AOCS Cd 8-53	9	19.65	0.54	3	0.6	
	SafTest Peroxide Test	9	19.19	2.19	11		97.2
Olive Oil 2	AOCS Cd 8-53	8	31.77	0.98	3	0.7	
	SafTest Peroxide Test	8	31.23	3.52	11		99.0
Olive Oil 3	AOCS Cd 8-53	8	41.13	0.80	2	1.7	
	SafTest Peroxide Test	8	39.77	4.18	10		96.4
Olive Oil 4	AOCS Cd 8-53	9	29.69	0.45	2	1.6	
	SafTest Peroxide Test	9	28.45	2.52	9		92.3
Olive Oil 5	AOCS Cd 8-53	9	36.62	1.16	3	9.8	
	SafTest Peroxide Test	9	43.86	2.98	7		111.1
Olive Oil 6	AOCS Cd 8-53	7	14.35	0.35	2	0.0	
	SafTest Peroxide Test	7	14.33	1.02	7		100.0
Olive Oil 7	AOCS Cd 8-53	10	22.91	0.82	4	1.9	
	SafTest Peroxide Test	10	24.26	3.05	13		105.4
Olive Oil 8	AOCS Cd 8-53	10	18.32	0.26	1	0.6	
	SafTest Peroxide Test	10	17.90	0.29	2		92.1
Olive Oil 9	AOCS Cd 8-53	10	4.04	0.07	2	1.3	
	SafTest Peroxide Test	10	3.09	0.06	2		79.5
Olive Oil 10	AOCS Cd 8-53	10	1.75	0.14	8	0.2	
	SafTest Peroxide Test	10	1.59	0.02	1		90.6
Olive Oil 11	AOCS Cd 8-53	10	9.01	0.14	2	1.5	
	SafTest Peroxide Test	10	7.93	0.19	2		88.7

[a]Number of replicates

[b]Recoveries calculated as a % of the AOAC Cd 8-53 mean. Average recovery = 95.7 %

Table 11: Matrix Study: Meat Meals and Fish Meal (1)

Matrix	Method	N[a]	Mean Peroxide, meq/kg	s_r	RSD_r, %[a]	t value	Recovery, %[b]
Lamb Meal #5	AOCS Cd 8-53	10	4.44	0.19	4	0.6	89.9
	SafTest Peroxide Test	10	3.98	0.14	4		
Fish Meal #1	AOCS Cd 8-53	10	5.81	0.44	8	1.0	86.3
	SafTest Peroxide Test	10	5.09	0.09	2		
Fish Meal #2	AOCS Cd 8-53	10	10.18	0.40	4	0.7	95.1
	SafTest Peroxide Test	10	9.69	0.20	2		
A Poultry Meal #2	AOCS Cd 8-53	10	0.50	0.02	4	0.02	94.0
	SafTest Peroxide Test	10	0.47	0.03	6		
Meat & Bone Meal #1	AOCS Cd 8-53	10	1.47	0.1	7	0.1	103.7
	SafTest Peroxide Test	10	1.55	0.04	3		
Meal #13	AOCS Cd 8-53	10	3.20	0.24	8	0.3	93.1
	SafTest Peroxide Test	10	2.98	0.05	2		
Meal #2	AOCS Cd 8-53	10	3.68	0.19	5	1.0	80.5
	SafTest Peroxide Test	10	2.96	0.10	3		
Poultry Meal #35	AOCS Cd 8-53	10	0.22	0.01	5	0.1	81.8
	SafTest Peroxide Test	10	0.18	0.03	17		
Poultry Meal #4	AOCS Cd 8-53	10	0.54	0.01	2	0.0	100.0
	SafTest Peroxide Test	10	0.54	0.04	7		
Poultry Meal #21	AOCS Cd 8-53	10	0.54	0.08	15	0.1	89.7
	SafTest Peroxide Test	10	0.48	0.03	7		

[a]Number of replicates. Note SafTest Peroxide Test represents unique replicates from the point of sampling. The AOCS Cd 8-53 tests were replicated from the point of titration, mitigating or eliminating sampling error, so the s_r values represent different error assessments.
[b]Recoveries calculated as a % of the AOAC Cd 8-53 mean. Average recovery = 91.5 %

Table 12: Matrix Study: Animal Fats and Oils (1)

Matrix	Method	N[a]	Mean Peroxide, meq/kg	s_r	RSD_r, %	t value	Recovery, %[b]
Poultry Fat #21	AOCS Cd 8-53	10	0.894	0.029	3	0.1	
	SafTest Peroxide Test	10	0.850	0.014	2		94.9
Poultry Fat #1	AOCS Cd 8-53	10	4.408	0.104	2	0.8	
	SafTest Peroxide Test	10	3.866	0.142	4		87.9
Poultry Fat #2	AOCS Cd 8-53	10	7.998	0.331	4	1.6	
	SafTest Peroxide Test	10	6.845	0.139	2		85.6
Animal Fat #19 Tallow/Saturated Fat	AOCS Cd 8-53	10	1.448	0.186	13	1.7	
	SafTest Peroxide Test	10	1.150	0.040	4		79.9
Poultry Fat #4	AOCS Cd 8-53	10	0.582	0.043	7	0.1	
	SafTest Peroxide Test	10	0.663	0.032	5		115.2
Turkey Fat #7	AOCS Cd 8-53	10	9.755	0.262	3	1.4	
	SafTest Peroxide Test	10	8.696	0.205	2		114.1
Poultry Fat #35	AOCS Cd 8-53	10	0.360	0.040	11	0.1	
	SafTest Peroxide Test	10	0.435	0.030	7		122.3
Poultry Meal #3	AOCS Cd 8-53	10	4.061	0.201	5	0.7	
	SafTest Peroxide Test	10	3.573	0.081	2		87.9
Butter #1 Saturated Fat, 35%	AOCS Cd 8-53	10	0.750	0.045	6	0.1	
	SafTest Peroxide Test	10	0.687	0.028	4		92.0
Turkey Fat #7	AOCS Cd 8-53	10	1.164	0.113	10	0.2	
	SafTest Peroxide Test	10	1.036	0.041	4		89.9

[a]Number of replicates

[b]Recoveries calculated as a % of the AOAC Cd 8-53 mean. Average recovery = 105.2 %

Table 13. Independent Laboratory Matrix Study: Oils, Butter and Tallow (1)

Matrix/Lab ID	Method	N	Peroxide, meq/kg [a]	s_r	RSD, %	Bias[b]	Recovery[c], %
Extra Virgin Olive Oil	AOCS Cd 8b-90	8	16.18	2.03	12.4	1.97	112.2
	SafTest Peroxide Test	8	18.15	1.29	7.1		
Soybean Oil	AOCS Cd 8b-90	8	1.88	0.12	6.5	-0.46	75.7
	SafTest Peroxide Test	8	1.42	0.03	5.5		
Clarified Butter	AOCS Cd 8b-90	8	2.48	0.19	7.8	-1.29	47.9
	SafTest Peroxide Test	8	1.19	0.13	11.0		
Beef Tallow	AOCS Cd 8b-90	8	<0.10	NC[d]	NC[d]	NC[d]	NC[d]
	SafTest Peroxide Test	8	<5.3	NC[d]	NC[d]		

[a] Mean Peroxide Result, meq/kg
[b] Bias calculated as SafTest Peroxide Test result minus AOCS Cd 8b-90 result
[c] Recovery of the SafTest Peroxide Test expressed as a % of AOCS Cd 8b-90 result
[d] Not calculated

Table 14. Independent Laboratory Matrix Study: Snack Products and Chicken Meal (1)

Matrix/Lab ID	Method	N	Peroxide, meq/kg[a]	Peroxide, meq/kg fat [b]	s_r	RSD, %	Bias[c]	Recovery[d], %
Crackers	AOCS Cd 8b-90	8		4.45	0.72	16.3	2.16	
	SafTest Peroxide Test	8	0.629	2.29[e]	0.32	14.0		51.5
Baked Corn Scoops	AOCS Cd 8b-90	8		7.47	0.40	5.4	3.33	
	SafTest Peroxide Test	8	0.864	10.8[e]	0.35	3.2		144.6
Potato Chips	AOCS Cd 8b-90	8		3.14	0.63	19.4	-0.68	
	SafTest Peroxide Test	8	0.9087	2.46[eb]	0.16	6.6		78.4
Chicken Meal	AOCS Cd 8b-90	8		21.25	1.44	6.8	3.00	
	SafTest Peroxide Test	8	3.36	24.25[e]	0.58	2.4		114.2

[a] Mean Peroxide Result, meq/kg, n=8.
[b] SafTest Peroxide results calculated from as-is basis. AOAC Cd 8b-90 results determined on extracted fat.
[c] Bias calculated as SafTest Peroxide Test, % Fat Basis result minus AOCS Cd 8b-90 result.
[d] Recovery of the SafTest Peroxide Test, % Fat Basis expressed as a % of AOCS Cd 8b-90 result.
[e] Calculated from As-Is Basis result divided by % fat as decimal fraction. % fat determined using SafTest Percent Fat Test.

Table 15. Independent Laboratory Matrix Study: Spike Recovery from Meat & Bone Meal and Fish Meal (1)

Matrix/Lab ID	Method	N	Peroxide, meq/kg [a]	s_r	RSD, %	Bias[b]	Recovery[c], %
Meat & Bone Meal	Theoretical	8	0.948				
	SafTest Peroxide Test	8	1.006	0.049	4.85	0.058	106.2
Fish Meal	Theoretical	8	1.489				
	SafTest Peroxide Test	8	1.432	0.075	5.28	-0.057	96.17

[a] Mean Peroxide Result, meq/kg
[b] Bias calculated as SafTest Peroxide Test result minus AOCS Cd 8b-90 result
[c] Recovery of the SafTest Peroxide Test expressed as a % of AOCS Cd 8b-90 result

REFERENCES CITED

1. Gordon, V.C., Rainey, C.C., and Studmire, W.C., Validation Study of the SafTest Peroxide Test Kit for the Measurement of the Peroxide Content of Oils, Tallows, Meat Meals, Potato Chips and Grain Based Snacks, AOAC *Performance Tested Methods*℠ certification number 102001
2. Official Methods and Recommended Practices of the AOCS (1997) 5th Ed., The American Oil Chemists' Society, Champaign, IL. Method Cd 8-53.
3. Official Methods of Analysis (2016) 20th ed. AOAC INTERNATIONAL, Rockville, MD, Method AOAC 965.33, http://eoma.aoac.org/.
4. Official Methods and Recommended Practices of the AOCS (2017) 7th Ed., The American Oil Chemists' Society, Urbana, IL. Method Cd 8b-90.