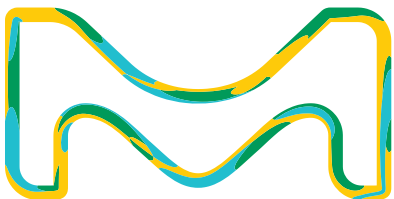


# Inside Meat Assurance

Rapid Testing for  
Quality & Compliance



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# 1. Meat and Meat Products Testing

Every cut of meat tells a story — of texture, taste, and trust. Beneath its flavor and color lies a complex chemistry that defines quality and determines safety. Nitrites stabilize color and suppress microbial growth, while chlorides influence flavor and moisture balance. Phosphates enhance juiciness and texture, hydroxyproline reflects structural protein content, and pH governs freshness, tenderness, and shelf life. Together, these parameters shape the sensory attributes, stability, and authenticity of meat. Understanding and monitoring them is essential for maintaining product integrity, meeting regulatory standards, and sustaining consumer confidence.

Since October 9, 2025, meat and meat products have to be monitored under new nitrite limits set by EU Commission Regulation (EU) 2023/2108, amending Annex II of Regulation (EC) No 1333/2008. These updated limits mark a new chapter in food safety, redefining how meat is assessed for both quality and compliance. By setting stricter thresholds, the regulation seeks to reduce the formation of nitrosamines and reinforce consumer protection across every category of meat products. This development highlights the need for analytical methods that are not only accurate but also fast, reliable, and adaptable to routine control and compliance testing.

To address these requirements, a series of rapid analytical methods has been developed to support the determination of key quality and safety parameters in meat and processed products. Using photometric and reflectometric techniques, these methods provide accurate quantification of nitrite, chloride, phosphorus, hydroxyproline, and pH across diverse matrices. Implemented through the Spectroquant®, Reflectoquant®, and MQuant® test kits, reagents and test strips, they enable reliable assessment of compositional consistency, additive concentration, and regulatory compliance. Together, these methods establish a foundational framework for meat analysis, ensuring that results are reproducible, traceable, and aligned with modern food safety and quality standards.

## Test Kits and Test Strips for Meat Analysis

Test kits and test strips provide fast, simple, and dependable solutions for the analysis of key quality and safety indicators in meat and processed products. They provide an efficient and accessible means to determine key parameters such as nitrite, chloride, phosphorus, hydroxyproline, and pH, supporting both process monitoring and regulatory compliance.

Photometric methods, used with Spectroquant® test kits, rely on specific color reactions that form measurable complexes proportional to analyte concentration. These tests are performed directly on prepared extracts using Spectroquant® photometers, e.g. from the Prove series, offering accuracy and reproducibility. The tests can also be used on instruments from other manufacturers after respective method calibration.

Reflectometric analysis with Reflectoquant® test strips and the handheld reflectometer RQflex® 20 enables fast, on-site testing, making it ideal for routine in-process verification. Visual methods using MQuant® test strips complement these approaches by allowing rapid semi-quantitative determination of pH and related parameters through direct color comparison.

Together, these analytical tools streamline quality control workflows in laboratories and production environments. They provide reliable data for assessing composition, verifying additive content, and maintaining compliance, helping ensure that meat products consistently meet safety and quality standards.

# 2. Method Overview

**Table 1.** Rapid test kits and test strips for meat and meat product testing

Parameter	Cat. No.	Measuring Range	Method principle	Page
Nitrite	1.14547	0.03–2.30 mg/L NO <sub>2</sub> <sup>-</sup>	Photometric with Spectroquant® Test Kit	4 & 7
	1.14776	0.007–3.28 mg/L NO <sub>2</sub> <sup>-</sup>	Photometric with Spectroquant® Test Kit	4 & 7
	1.16973	0.5–25.0 mg/L NO <sub>2</sub> <sup>-</sup>	Reflectometric with Reflectoquant® Test strips	9
	1.10007	2–80 mg/L NO <sub>2</sub> <sup>-</sup>	Colorimetric with MQuant® Test strips	10
Chloride	1.14730	5–125 mg/L Cl <sup>-</sup>	Photometric with Spectroquant® Test Kit	11
	1.14897	2.5–250 mg/L Cl <sup>-</sup>	Photometric with Spectroquant® Test Kit	11
Hydroxyproline		0.000–1.000 g/100 g	Photometric with reagents	14
Phosphorus	1.14543	0.05–5.00 mg/L P	Photometric with Spectroquant® Test Kit	21
	1.14729	0.5–25.0 mg/L P	Photometric with Spectroquant® Test Kit	21
	1.14848	0.005–5.00 mg/L P	Photometric with Spectroquant® Test Kit	22
	1.10428	10–500 mg/L PO <sub>4</sub> <sup>3-</sup>	Colorimetric with MQuant® Test strips	22
		0.000–2.500 g/100 g P <sub>2</sub> O <sub>5</sub>	Photometric with reagents	18
pH	1.09632	pH 5.2–7.2	Colorimetric with MQuant® pH indicator strips	25

## 3. Nitrite

Nitrite, in the form of sodium nitrite (E250) and potassium nitrite (E249), is widely used in meat processing to ensure microbiological safety, stabilize cured color, and enhance flavor.<sup>1</sup> Its inhibitory effect against *Clostridium botulinum* is considered critical for consumer protection, and for this reason nitrite has been authorized as a food additive under European legislation, including Regulation (EC) No 1333/2008 and its subsequent amendments.

Despite these important functions, nitrites are associated with toxicological concerns. Under specific conditions, they may give rise to *N*-nitroso compounds, a class of substances classified by the International Agency for Research on Cancer (IARC) as probable human carcinogens.<sup>1</sup> Regulation of nitrite use, therefore, emphasizes maintaining a balance between microbiological safety and minimizing nitrosamine formation. This balance is reflected in strict maximum levels established by the European Union<sup>2</sup> and other international authorities which continue to regulate the use of nitrites in meat and meat products.

Several analytical techniques, including electrochemical and chromatographic methods, have been explored for nitrite determination, yet spectrophotometric approaches remain predominant due to their simplicity, cost-effectiveness, and suitability for routine analysis.<sup>3,4</sup> The photometric Griess nitrite test, in which extracted nitrite is converted into a stable azo dye, provides a reliable reference for routine quality control and regulatory monitoring of nitrite in meat products.

### 3.1 Nitrite in Dried Meat

#### 3.1.1 Method

Nitrite is extracted from dried meat using a potassium hydrogen phthalate solution. Then in acidic solution, nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically.

#### 3.1.2 Measuring Range

Spectroquant® Nitrite Cell Test (1.14547):	Test kit	0.03–2.30 mg/L NO <sub>2</sub> <sup>-</sup>
	Method	0.60–46.0 mg/kg NO <sub>2</sub> <sup>-</sup>
		0.90–69.0 mg/kg NaNO <sub>2</sub>
Spectroquant® Nitrite Test (1.14776):	Test kit	0.007–3.28 mg/L NO <sub>2</sub> <sup>-</sup>
	Method	0.14–65.6 mg/kg NO <sub>2</sub> <sup>-</sup>
		0.21–98.4 mg/kg NaNO <sub>2</sub>

#### 3.1.3 Reagents, Instruments and Materials

##### Reagent & Test Kits

- Spectroquant® Nitrite Cell Test (1.14547) or
- Spectroquant® Nitrite Test (1.14776)
- Potassium hydrogen phthalate for analysis (1.04874)
- MQuant® pH-indicator strips pH 0–6.0 (1.09351)
- Sulfuric acid 0.5 mol/L Titripur® (1.09072)
- Sodium hydroxide solution 1 mol/L Titripur® (1.09137)
- Water for analysis (1.16754)

##### Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (1.73026)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (1.73027)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (1.73028)
- Spectroquant® Colorimeter Move 100 (1.73632)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

## Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

## Instrument Accessories

- Rectangular cells 10 mm (**1.14946**) or
- Rectangular cells 20 mm (**1.14947**) or
- Rectangular cells 50 mm (**1.14944**)

**Note:** Rectangular cells are only necessary if the Spectroquant® Nitrite test **1.14776** is used.

## Other Reagents and Accessories

- Analytical balance
- Volumetric flask, 100 mL
- Schliff Erlenmeyer flask, 250 mL
- Knife or scissors
- Mixer or electric coffee mill
- Centrifuge
- Standard laboratory glassware (e.g., glass beakers) and pipettes
- Folded filters

### 3.1.4 Analytical Procedure

#### Reagent Preparation

Weigh 2.0 g potassium hydrogen phthalate and transfer it into a 100 mL volumetric flask. Fill up to the mark using water for analysis.

#### Sample Preparation

Cut down the sample with a knife or with scissors and ground it in a mixer or an electric coffee-mill to a fine, fibrous powder. In a closed Schliff-Erlenmeyer flask stir 5 g of the grounded product with 95 mL water for analysis and 5 mL potassium hydrogen phthalate solution 2% for 2 hours. Then separate the suspension in the centrifuge at 4000 turns per minute for 15 minutes. Filter the supernatant solution through a folded filter (=pretreated sample).

#### Using Cat. No. 1.14547: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

#### Procedure

- Pipette 5.0 mL pretreated sample into a reaction cell, close the cell tightly, and shake **vigorously until the reagent is completely dissolved**.
- **Leave to stand for 10 min (reaction time)**, then measure the sample in the photometer.

#### Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

## Using Cat. No. 1.14776: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

### Procedure

- Pipette 5.0 mL pretreated sample into a test tube,
- Add 1 level microspoon (in the cap of the NO<sub>2</sub><sup>-</sup>-1 bottle) and shake **vigorously for 1 min until the reagent is almost completely dissolved. The pH must be in the range of pH 2.0–2.5.** Check with MQuant® pH-indicator strips. Adjust, If necessary, with sodium hydroxide solution or sulfuric acid.
- **Leave to stand for 10 min (reaction time)**, then fill the sample into the cell, and measure the sample in the photometer.

**Note:** For measurement in the 50 mm cell both the sample volume as well as the quantity of reagent NO<sub>2</sub><sup>-</sup>-1 must be doubled. Alternatively, the semi-microcell **Cat. No. 1.73502** can be used. It is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.

### Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- If a 50 mm cell is used, it is recommended to perform the reagent blank one time for each measurement series. To do this tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- After the reagent blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

### 3.1.5 Calculation

Nitrite content in mg/kg NO<sub>2</sub><sup>-</sup> = analysis value in mg/L NO<sub>2</sub><sup>-</sup> x 20

Nitrite content in mg/kg NaNO<sub>2</sub> = analysis value in mg/L NO<sub>2</sub><sup>-</sup> x 30

### 3.1.6 Analytical Quality Assurance

Analytical quality assurance (AQA is recommended before each measurement series.

To check the photometric measurement system (test reagent, measurement device, handling) and the mode of working, the nitrite standard solution (see section 5 of the respective test kit instruction) can be used.

Sample-dependent interferences (matrix effects) can be determined by means of standard addition.

To view additional notes, visit **[SigmaAldrich.com/qa-test-kits](https://www.sigmaaldrich.com/qa-test-kits)**.



## 3.2 Nitrite in Meat and Sausage Products

### 3.2.1 Method

After aqueous extraction and Carrez clarification the nitrite ions react in acidic solution with sulfanilic acid to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically.

### 3.2.2 Measuring Range

Spectroquant® Nitrite Cell Test (1.14547):	Test kit	0.03–2.30 mg/L NO <sub>2</sub> <sup>-</sup>
	Method	0.60–46.0 mg/kg NO <sub>2</sub> <sup>-</sup>
		0.90–69.0 mg/kg NaNO <sub>2</sub>
Spectroquant® Nitrite Test (1.14776):	Test kit	0.007–3.28 mg/L NO <sub>2</sub> <sup>-</sup>
	Method	0.14–65.6 mg/kg NO <sub>2</sub> <sup>-</sup>
		0.21–98.4 mg/kg NaNO <sub>2</sub>

### 3.2.3 Reagents, Instruments and Materials

#### Reagent and Test Kits

- Spectroquant® Nitrite Cell Test (1.14547) or
- Spectroquant® Nitrite Test (1.14776)

#### Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (1.73026)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (1.73027)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (1.73028)
- Spectroquant® Colorimeter Move 100 (1.73632)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

#### Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (Y.11086) to transfer your data into an existing LIMS system.

#### Instrument Accessories

- Rectangular cells 10 mm (1.14946) or
- Rectangular cells 20 mm (1.14947) or
- Rectangular cells 50 mm (1.14944)

**Note:** Rectangular cells are only necessary if the Spectroquant® Nitrite test 1.14776 is used.

#### Other Reagents and Accessories

- MQuant® pH-indicator strips pH 0–6.0 (1.09531)
- Carrez Clarification (1.10537)
- Sodium hydroxide solution (1.09137)
- Water for analysis (1.16754)
- Standard laboratory glassware (e.g. Erlenmeyer flasks) and pipettes
- Analytical balance
- Ultra Turrax
- pH-Meter
- Heating bath
- Folded filters

### 3.2.4 Analytical Procedure

#### Sample Preparation

In an Erlenmeyer-flask weigh exactly 10 g of the sample and mix with about 80 mL water for analysis. With the Ultra-Turrax high speed blender homogenize the mixture for 60 seconds. With 50 mL hot water for analysis rinse the shaft of the homogenizer into the flask, adding it to the mixture. Then adjust the pH-value of the solution to 7–7.2 with sodium hydroxide solution 1 mol/L, using the pH-meter, and heat for 15 minutes in a bath of boiling water, while occasionally shaking. After cooling down to room temperature quantitatively transfer the prepared sample into a 200 mL standard volumetric flask and successively mix it with 2 mL Carrez solution-1 and -2 at a time. With rich in connective tissue products use 4 mL Carrez solution-1 and -2 respectively. Shake after each addition. Then fill up to volume with water for analysis and, after mixing, filter through a folded filter. Discard the first filtrate and use the remaining, clear filtrate for the determination (=pretreated sample).

#### Using Cat. No. 1.14547: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

##### Procedure

- Pipette 5.0 mL pretreated sample into a reaction cell, close the cell tightly, and shake **vigorously until the reagent is completely dissolved**.
- **Leave to stand for 10 min (reaction time)**, then measure the sample in the photometer.

##### Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

#### Using Cat. No. 1.14776: Procedure and Measurement

For more information on the measurement see the packaging insert of the test.

##### Procedure

- Pipette 5.0 mL pretreated sample into a test tube.
- Add 1 level microspoon (in the cap of the NO<sub>2</sub>-1 bottle) and shake **vigorously for 1 min until the reagent is almost completely dissolved. The pH must be in the range of pH 2.0–2.5**. Check with MQuant® pH-indicator strips. Adjust, If necessary, with sodium hydroxide solution or sulfuric acid.
- **Leave to stand for 10 min (reaction time)**, then fill the sample into the cell, and measure the sample in the photometer.

**Note:** For measurement in the 50 mm cell both the sample volume as well as the quantity of reagent NO<sub>2</sub>-1 must be doubled. Alternatively, the semi-microcell **Cat. No. 1.73502** can be used. It is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.



## Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- If a 50 mm cell is used, it is recommended to perform the reagent blank one time for each measurement series. To do this tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- After the reagent blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

### 3.2.5 Calculation

Nitrite content in mg/kg  $\text{NO}_2^-$  = analysis value in mg/L  $\text{NO}_2^-$  x 20

Nitrite content in mg/kg  $\text{NaNO}_2^-$  = analysis value in mg/L  $\text{NO}_2^-$  x 30

### 3.2.6 Analytical Quality Assurance

Analytical Quality Assurance (AQA) is recommended before each measurement series. To check the photometric measurement system (test reagent, measurement device, handling) and the mode of working, the nitrite standard solution (see section 5 of the respective test instruction) can be used.

Sample-dependent interferences (matrix effects) can be determined by means of standard addition.

To view additional notes, visit [SigmaAldrich.com/qa-test-kits](https://www.sigmaaldrich.com/qa-test-kits).

## 3.3 Nitrite in Meat Products

### 3.3.1 Method

In the presence of an acidic buffer nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl) ethylenediamine to form a red-violet azo dye that is determined reflectometrically.

### 3.3.2 Measuring Range

Reflectoquant® Nitrite Test (1.16973):		0.5–25.0 mg/L $\text{NO}_2^-$
	Test kit	(0.2–7.6 mg/L $\text{NO}_2^-$ – N)
	Method	1.5–75 mg/kg $\text{NO}_2^-$

### 3.3.3 Reagents, Instruments and Materials

#### Test/Reagents Kit

- Reflectoquant® Nitrite Test (1.16973)

#### Instrument(s) & Devices

- Reflectometer RQflex® 20, Reflectoquant® (1.17246)

## Other Reagents and Accessories

- MQuant® Nitrite Test 2–80 mg/L NO<sub>2</sub><sup>-</sup> (0.6–24 mg/L NO<sub>2</sub>-N) (**1.10007**)
- Distilled water (e.g., from a suitable Milli-Q® system) or water for analysis (**1.16754**)
- Balance
- Beaker
- Blender
- Heating plate
- Folded filter

## Products for AQA

- RQcheck set for RQflex® 20 Reflectometer (**1.17247**)
- Recalibration Set for RQflex® 20 Reflectometer (**1.16954**)
- Nitrite standard solution Certipur®, 1000 mg/L NO<sub>2</sub><sup>-</sup> (**1.19899**)

### 3.3.4 Analytical Procedure

#### Sample Preparation

- Weigh approximately 50 g chopped meat exactly into a beaker, add 150 mL distilled water and homogenize in a blender.
- Heat this mixture to 80 °C and filter through a folded filter. Allow the solution to cool down to room temperature before starting the analysis.
- Check the nitrite content with the MQuant® Nitrite Test. Samples containing more than 25.0 mg/L NO<sub>2</sub><sup>-</sup> must be diluted with distilled water.

#### Measurement

- Press the START button of the reflectometer **and - this is imperative - at the same time** immerse both reaction zones of the test strip in the pretreated sample (15–30 °C) for 2 seconds.
- Carefully allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel.
- Immediately insert the strip all the way into the strip adapter with the reaction zones facing the display.
- After the end of the reaction time (15sec), read off the result from the display in mg/L NO<sub>2</sub><sup>-</sup>. The result is automatically stored.

#### Notes on the measurement:

For detailed operating procedures, consult the operating instructions for RQflex® instrument and the package insert of the Reflectoquant® Nitrite Test.

### 3.3.5 Calculation

$$\text{Nitrite content [mg/kg]} = \frac{\text{Measured value [mg/L]} \times 150 \text{ [mL]}}{\text{Weight of sample [g]}}$$

### 3.3.6 Analytical Quality Assurance

Analytical Quality Assurance (AQA) is recommended before each measurement series. Check the instrument using the RQcheck. If RQcheck failed, perform a recalibration using the recalibration set and repeat the RQcheck. For more details see RQflex® 20 manual.

To check test strips, test reagent, measurement device, and handling (recommended before each measurement series): Dilute the nitrite standard solution with distilled water to 10.0 mg/L NO<sub>2</sub><sup>-</sup> and analyze as described in the section measurement of the instructions for use of the Reflectoquant® Nitrite Test.

## 4. Chloride

Sodium chloride is one of the most widely used additives in meat and sausage production, where it contributes to flavor, texture, protein solubilization, water-holding capacity, and microbial stability. Beyond its technological roles, salt levels in processed meats are also of public health importance, as excessive sodium intake is strongly associated with hypertension, cardiovascular disease, and other chronic health conditions.<sup>5</sup> The World Health Organization (WHO) has consistently emphasized the need to reduce dietary sodium intake, and the meat industry has been under increasing regulatory and consumer pressure to monitor and reduce salt levels in processed products.<sup>6</sup> Accurate determination of sodium chloride is therefore essential not only for product quality but also for compliance with labeling requirements and dietary health guidelines.

Although the analytical target is sodium chloride, official reference methods quantify chloride ions as the marker for salt content. The Association of Official Analytical Chemists specifies chloride determination as the standard approach, with AOAC 971.27 (Chloride in Meat Products) and related methods providing validated protocols for this purpose.<sup>7</sup> Chloride determination remains the regulatory benchmark for salt analysis in meat and sausage products, ensuring consistency and comparability across laboratories.<sup>6,7</sup>

Spectrophotometric determination of chloride offers a practical alternative to classical titrimetric methods such as the Mohr and Volhard procedures. Compared with titration, spectrophotometric assays provide improved sensitivity, reduced sample handling, and faster throughput, making them highly suitable for routine laboratory and industrial quality control. Their use enables accurate monitoring of sodium chloride addition in meat matrices, thereby ensuring compliance with food safety regulations, supporting product standardization, and contributing to ongoing efforts to reduce dietary sodium exposure.<sup>6,7</sup>

### 4.1 Chloride in Meat and Sausage Products

#### 4.1.1 Method

After aqueous extraction and Carrez clarification the chloride ions from the sample react with mercury(II) thiocyanate to form slightly dissociated mercury(II) chloride. The thiocyanate released in the process in turn reacts with iron(III) ions to form red iron(III) thiocyanate that is determined photometrically.

#### 4.1.2 Measuring Range

<b>Spectroquant® Chloride Cell Test (1.14730):</b>	Test kit	5–125 mg/L Cl <sup>-</sup>
	Method	1.65–41.25 g/kg NaCl
<b>Spectroquant® Chloride Test (1.14897):</b>	Test kit	2.5–250 mg/L Cl <sup>-</sup>
	Method	0.825–82.5 g/kg NaCl

#### 4.1.3 Reagents, Instruments and Materials

##### Reagents & Test Kits

- Spectroquant® Chloride Cell Test (1.14730) or
- Spectroquant® Chloride Test (1.14897)
- Sodium hydroxide solution 1 mol/L (1.09137)
- Carrez Clarification Kit (1.10537)
- Water for analysis (1.16754)

##### Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (1.73026)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (1.73027)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (1.73028)
- Spectroquant® Colorimeter Move 100 (1.73632)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

## Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

## Instrument Accessories

- Rectangular cells 10 mm (**1.14946**)

**Note:** Rectangular cells are only necessary if the Spectroquant® test **1.14897** is used.

## Other Reagents and Accessories

- Standard laboratory glassware (e.g. Erlenmeyer flasks) and pipettes
- Analytical balance
- Ultra Turrax
- pH-Meter
- Heating bath
- Folded filters

### 4.1.4 Analytical Procedure

#### Sample Preparation

In an Erlenmeyer flask weigh, exactly as to 1 mg, 10 g of the sample and mix with about 80 mL water for analysis. With the Ultra-Turrax homogenize the mixture for 60 s. With 50 mL hot water for analysis rinse the shaft of the homogenizer into the flask, adding it to the mixture. Then adjust the pH-value of the solution to 7–7.2 with sodium hydroxide solution 1 mol/L, using the pH-meter, and heat for 15 minutes in a bath of boiling water, while occasionally shaking.

After cooling down to room temperature quantitatively transfer the prepared sample into a 200 mL volumetric flask and successively mix it with 2 mL Carrez solution 1 and 2 at a time. With rich in connective tissue products use 4 mL Carrez solution 1 and 2 respectively. Shake after each addition. Then fill up to volume with water and, after mixing, filter through a folded filter. Discard the first filtrate, dilute the residual clear filtrate 1:10 with water for analysis in the volumetric flask and use it for determination (=pretreated sample).

#### Using Cat. No. 1.14897: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

#### Procedure (Measuring range 2.5–25.0 mg/L Cl<sup>-</sup>)

- Pipette 5.0 mL pretreated sample in a test tube
- Add 2.5 mL reagent Cl-1 with a pipette and mix.
- Add 0.50 mL reagent Cl-2 with a pipette and mix.
- **Leave to stand for exactly 1 min (reaction time)**, then fill the sample into a 10 mm cell, and measure **immediately** in the photometer.

#### Procedure (Measuring range 10–250 mg/L Cl<sup>-</sup>)

- Pipette 1.0 mL sample solution in a test tube
- Add 2.5 mL reagent Cl-1 with a pipette and mix.
- Add 0.50 mL reagent Cl-2 with a pipette and mix.
- **Leave to stand for exactly 1 min (reaction time)**, then fill the sample into a 10 mm cell, and measure **immediately** in the photometer.

## Measurement

- **The color of the measurement solution remains stable for only a short time.**
- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero adjustment, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

## Using Cat. No. 1.14730: Procedure and Measurement

For more information on the measurement see the packaging insert of the test.

### Procedure

- Pipette 0.50 mL reagent CI-1K into a reaction cell, close the cell, and mix.
- Add 1.0 mL pretreated sample with a pipette, close the cell, and mix.
- Measure the sample **immediately** in the photometer.

## Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

### 4.1.5 Calculation

Sodium chloride content in mg/kg NaCl = analysis value in mg/L Cl<sup>-</sup> x 330

### 4.1.6 Analytical Quality Assurance

Analytical quality assurance (AQA) is recommended before each measurement series.

To check the photometric measurement system (test reagent, measurement device, handling) and the mode of working, chloride standard solutions (see section 5 of the respective test kit instruction) or Spectroquant® CombiCheck 10 and 20 or 60, respectively can be used. Besides a **standard solution** with 25 mg/L Cl<sup>-</sup> (CombiCheck 10) or, respectively, 60 mg/L Cl<sup>-</sup> (CombiCheck 20), or respectively 125 mg/L Cl<sup>-</sup> (CombiCheck 60) these articles also contain an **addition solution** for determining sample-dependent interferences (**matrix effects**).

To view additional notes, visit **[SigmaAldrich.com/qa-test-kits](https://www.sigmaaldrich.com/qa-test-kits)**.

## 5. Hydroxyproline

Hydroxyproline is a non-essential amino acid predominantly localized within collagen molecules,<sup>8</sup> thereby serving as a specific biochemical marker for collagen determination in meat and meat products. Collagen, the major structural protein of animal connective tissue, influences both sensory and nutritional attributes of processed meats, with elevated levels being associated with undesirable texture and reduced concentrations of essential amino acids, which are critical indicators of product quality.<sup>8</sup> Since hydroxyproline constitutes up to 10% of the collagen structure, its quantification provides a reliable estimate of total collagen content.<sup>9</sup> Owing to its scarcity in proteins other than collagen, hydroxyproline determination is widely applied as a standardized method for assessing meat quality and for detecting adulteration with low-value raw materials.<sup>9</sup>

The official procedure described in the German Food and Feed Code §64 LFGB 06.00–8 is based on a colorimetric determination of hydroxyproline. This standardized method enables accurate quantification of hydroxyproline, and thereby collagen, in a variety of meat matrices, supporting regulatory compliance, compositional analysis, and product labeling.

**Note:** Pursuant to the valid copyright regulations the method provided below contains only a rough description of the content in the official method followed by a detailed description of the specific measurement procedure with the Spectroquant® Prove (plus) Spectrophotometers. A detailed description of the method specific handling steps can be found in the official method of the German Food and Feed Code §64 LFGB 06.00–8.<sup>10</sup>

### 5.1 Hydroxyproline in Meat, Meat Products and Sausage Products (According to German Food and Feed Code §64 LFGB 06.00–8)

#### 5.1.1 Method

The Hydroxyproline content is determined after acid hydrolysis of the sample followed by the separation of the fat content and oxidation with Chloramine T. The oxidized form of Hydroxyproline reacts with 4-(Dimethylamino) benzaldehyde to form a red-colored product that is measured photometrically at 430 nm. This method is based on the official method of the German Food and Feed Code §64 LFGB 06.00–8<sup>10</sup> and describes the determination of Hydroxyproline in meat, meat products and sausages.

#### 5.1.2 Measuring Range

Method 2538	Hydroxyproline Meat §64 LFGB 06.00–8
	0.000–1.000 g/100 g

#### 5.1.3 Reagents, Instruments and Materials

##### Reagents

- Chloramine T Trihydrate for analysis (1.02426)
- Hydrochloric acid solution 6.0 mol/L (6.0 N) (1.43007)
- Petroleum benzene boiling range 40–60 °C SupraSolv® (1.01772)
- Citric acid monohydrate for analysis EMSURE® (1.00244)
- Sodium hydroxide pellets for analysis EMSURE® (1.06498)
- Sodium acetate anhydrous for analysis EMSURE® (1.06268)
- Perchloric acid 60% for analysis EMSURE® (1.00518)
- 1-Propanol for analysis EMSURE® (1.00997)
- 2-Propanol for analysis EMSURE® (1.09634)



## Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Prove series.

## Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

## Instrument Accessories

- Rectangular cells 10 mm (**1.14946**)

## Other Reagents and Accessories

- L-Hydroxyproline for biochemistry (optional, for AQA)
- DURAN® Laboratory bottles
- Round-bottomed flask or Erlenmeyer flask with NS and return condenser
- Test tube, closable, 10–15 mL
- Heating block, drying cabinet or heating plate
- Water bath (temperature controllable)
- Vacuum
- Funnel
- Folded filter, diameter 15 cm
- Volumetric flasks, 50 mL, 100 mL
- Standard laboratory glassware (e.g., glass beakers) and pipettes
- Analytical balance

### 5.1.4 Analytical Procedure

#### Preparation of Solutions

- **Buffer solution pH 6.8:** The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–8.<sup>10</sup>
- **Oxidizing reagent:** The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–8.<sup>10</sup>
- **Color reagent solution:** The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–8.<sup>10</sup>

#### Sample Preparation

Homogenize sample.

#### Preparation of Measurement Solutions

Acid hydrolysis and fat separation

- Weigh approx. 2 g of the homogenized sample with an accuracy of 1 mg to a DURAN® laboratory bottle and follow the procedure according to German Food and Feed Code §64 LFGB 06.00–8, chapter 7.<sup>10</sup>
- Note the sample weight.
- Use filtrate for the preparation of the measurement solution.

## Hydroxyproline Determination

### Reagent blank

- Mix 0.100 mL of distilled water with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.

### Sample

- Mix 0.100 mL of the resulted filtrate after acid hydrolysis and fat extraction of the sample with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.

### Measurement

**Note:** It is advisable to measure the reagent blank and the sample using the same cell as the one used for the zero adjustment or else a cell with identical optical characteristics and an identical absorption (matched pair).

- Open the methods list (<Methods>) and select Method No. 2538 "Hydroxyproline Meat §64 LFGB 06.00–8".
- The instrument automatically prompts a "Zero adjustment".
- For the zero adjustment fill a clean and dry 10 mm rectangular cell with distilled water.
- After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically.
- Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- A window with an input field to enter the sample weight pops up.
- Enter the weight of the sample in grams (g), accurate to 0.001 grams (g), confirm with <OK> and click on <START> to switch to the measurement procedure.

**Note:** It is possible to enter a sample weight in a range of 0.010 to 5.000 g.

- Fill the prepared reagent blank into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) symbol appears behind the cue "Insert Reagent Blank".
- Confirm the measurement by clicking on <OK>.
- Finally fill the prepared sample solution into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) appears behind the cue "Insert Sample".
- Confirm the measurement by clicking on <OK>
- Read off the result in g/100 g and the absorption for the reagent blank ( $A_{RB}$ ) and the sample ( $A_{Sample}$ ) from the display.
- Tap the <START> button to start the measurement procedure for the next sample.

### 5.1.5 Analytical quality assurance

- The method can be checked using L-Hydroxyproline as standard substance.
- Prepare a stock solution with 600 mg/l resp. 60 mg/100 mL L-Hydroxyproline by dissolving 60.0 mg L-Hydroxyproline in approx. 80 mL distilled water. Transfer the solution completely to a 100 mL volumetric flask and fill up to the mark with distilled water.
- Dilute the stock solution to 4.8 mg/100 mL L-Hydroxyproline by pipetting 4 mL of the 60 mg/100 mL L-Hydroxyproline stock solution into a 50 mL measuring flask and fill up to the mark with distilled water.
- Mix 0.100 mL of the prepared standard solution with 5 mL Oxidizing reagent in a closable test tube and incubate for 20 min at room temperature.
- Add 2 mL of Color reagent, close the tube, mix and incubate in a water bath at 60 °C for 15 min.
- Cool down the vessel to room temperature with running water within 3 min.
- Incubate for 30 min at room temperature.
- Measure this solution versus a reagent blank as described in the section "Measurement". Hereby enter a weight of 2.00 g.

**Note:** Due to the different sample preparation procedure and determination procedure of the standard solution compared to a sample analysis it is necessary to recalculate the displayed result manually as follows:

$$\text{Measured Concentration standard} \left[ \frac{\text{mg}}{100 \text{ mL}} \right] = \text{Displayed result [g/100 g]} \cdot \frac{F_1}{F_2} = \text{Displayed result [g/100 g]} \cdot \frac{1000}{50}$$

$$\text{Measured Concentration standard} \left[ \frac{\text{mg}}{100 \text{ mL}} \right] = \text{Displayed result [g/100 g]} \cdot 20$$

Where,  $F_1 = 1000$  = recalculation g/100 g to mg/100 mL  
 $F_2 = 50$  = Factor sample preparation for real sample

### Adjustment

- In case of significant deviations in the method control procedure the preprogrammed factor of 10.55 or the current factor used in the calculation of the displayed results can be adjusted by the user.
- The corrected factor must be recalculated as follows:  
**Factor corrected = Current factor x (target value standard / measured and recalculated value standard)**
- To edit the preprogrammed factor, select method 2538 from <Methods>.
- Close the window for the "Zero adjustment" by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list "FACTORS".
- Tap on the input field "Factor", enter the corrected factor and confirm by clicking on <OK>.
- Close the window for the "Zero adjustment" by clicking on <X>.
- For the next measurement restart the method by selecting the method anew from <Methods>.

### Note:

- To find the used factor, select Method 2538 from <Methods>.
- Close the window for the "Zero adjustment" by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list "FACTORS".

## 6. Phosphorus

Phosphorus is an essential nutrient with critical roles in cellular energy metabolism, signal transduction, phosphorylation reactions, and the mineralization of bones and teeth.<sup>11</sup> Major dietary sources include foods of animal origin such as milk, dairy products, meat, poultry, and fish, with additional contributions from cereals and legumes.<sup>12</sup> Beyond naturally occurring phosphorus, inorganic phosphates (E 338–341, E 343, E 450–452) derived from food additives constitute a significant and increasing proportion of total intake, particularly through processed and convenience foods. In the meat industry, food-grade phosphates are widely applied to enhance water-holding capacity, emulsification, and color stability, thereby improving product quality.<sup>16</sup> It is estimated that up to half of daily phosphorus intake in Western diets originates from such additive-derived “hidden phosphorus”.<sup>13</sup> Unlike protein-bound phosphorus, which is absorbed at about 60% efficiency, inorganic phosphate salts from additives are almost completely absorbed.<sup>14</sup>

Excessive phosphorus intake has been linked to cardiovascular morbidity and mortality, especially in individuals with chronic kidney disease, making it a broader public health concern. The Scientific Committee for Food established an Acceptable Daily Intake (ADI) for phosphates, expressed as phosphorus, of 40 mg/kg body weight per day.<sup>15</sup> Additive-derived phosphates pose particular concern because they dissociate readily without enzymatic hydrolysis, unlike organic phosphorus in animal- and plant-derived foods. Plant phosphorus, mostly bound to phytates, has a bioavailability of only 20–30% due to the absence of phytase in humans.<sup>15</sup> This higher bioavailability of additive phosphorus underscores the need for monitoring intake and exposure, particularly in vulnerable populations.

Accurate quantification of phosphorus in meat and sausage products is therefore necessary to ensure regulatory compliance, dietary exposure assessment, and quality control. Photometric determination using the phosphorus molybdenum blue method subsequent to fusion melting provides a robust and sensitive approach for total phosphorus measurement in complex meat matrices.

### 6.1 Total Phosphorus in Meat and Meat Products (According to German Food and Feed Code §64 LFGB 06.00–9)

**Note:** Pursuant to the valid copyright regulations this application note contains only a rough description of the content of the official method followed by a detailed description of the specific measurement procedure with the Spectroquant® Prove Spectrophotometers. A detailed description of the method specific handling steps can be found in the official method of the German Food and Feed Code §64 LFGB 06.00–9.<sup>16</sup>

#### 6.1.1 Method

The total phosphorus content in meat and meat products is determined after dry ashing followed by acid hydrolysis. The prepared sample reacts with ammonium vanadate and ammonium heptamolybdate to form orange yellow molybdovanado-phosphoric acid that is measured photometrically at 430 nm.

This method is based on the official method of the German Food and Feed Code §64 LFGB 06.00–9<sup>16</sup> and describes the determination of the total phosphorus in meat and meat products.

#### 6.1.2 Measuring Range

Method 2533	Phosphorus Meat §64 LFGB 06.00–9 0.000–2.500 g/100 g P <sub>2</sub> O <sub>5</sub>
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#### 6.1.3 Reagents, Instruments and Materials

##### Reagents

- Nitric acid 65% for analysis EMSURE® (1.00452)
- Ammonium monovanadate GR for analysis (1.01226)
- Ammonium heptamolybdate tetrahydrate GR for analysis (1.01182)
- Phosphate standard solution traceable to SRM from NIST, KH<sub>2</sub>PO<sub>4</sub> in H<sub>2</sub>O 1000 mg/L PO<sub>4</sub> Certipur® (1.19898)

## **Instrument(s) & Devices**

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Prove series.

## **Software for Data transfer**

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

## **Instrument Accessories**

- Rectangular cells 10 mm (**1.14946**)

## **Other Reagents and Accessories**

- Quartz or porcelain dishes
- Watch glass
- Muffle furnace
- Crucible tongs
- Exicator
- Water bath
- Folded filter, phosphate free
- Graduated cylinders, 10 mL, 20 mL
- Volumetric flasks, 100 mL, 1000 mL
- Standard laboratory glassware (e.g. glass beakers) and pipettes

## **Analytical balance**

### **6.1.4 Analytical Procedure**

#### **Reagent Preparation**

- Ammonium monovanadate solution – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–9.<sup>16</sup>
- Ammonium heptamolybdate solution – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–9.<sup>16</sup>
- Reagent solution – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00–9.<sup>16</sup>

#### **Sample Preparation**

According to German Food and Feed Code §64 LFGB 06.00–9.<sup>16</sup>

#### **Preparation of Measurement Solutions**

##### **Dry ashing**

- Weigh sample to a dish and follow the procedure according to German Food and Feed Code §64 LFGB 06.00–4.<sup>17</sup>
- Note the sample weight.

##### **Sample solution preparation**

- Hydrolyze the obtained ash according to the procedure of German Food and Feed Code §64 LFGB 06.00–9, chapter 7.3.<sup>16</sup>

## Phosphorus Determination

1. **Reagent blank** - Mix 2 mL of distilled water with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.
2. **Sample** - Mix 2 mL of the prepared Sample solution with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.

### Measurement

**Note:** It is advisable to measure the reagent blank and the sample using the same cell as the one used for the zero adjustment or else a cell with identical optical characteristics and an identical absorption (matched pair).

- Open the methods list (<Methods>) and select Method No. 2533 "Phosphorus Meat §64 LFGB 06.00–9".
- The instrument automatically prompts a "Zero adjustment".
- For the zero adjustment fill a clean and dry 10 mm rectangular cell with distilled water.
- After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically.
- Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- A window with an input field to enter the sample weight pops up.
- Enter the weight of the sample in grams (g), accurate to 0.001 grams (g), confirm with <OK> and click on <START> to switch to the measurement procedure.

**Note:** It is possible to enter a sample weight in a range of 0.010 to 10.000 g.

- Fill the prepared reagent blank into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) symbol appears behind the cue "Insert Reagent Blank".
- Confirm the measurement by clicking on <OK>.
- Finally fill the prepared sample solution into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) appears behind the cue "Insert Sample".
- Confirm the measurement by clicking on <OK>.
- Read off the result in g/100 g P<sub>2</sub>O<sub>5</sub> and the absorption for the reagent blank (A<sub>RB</sub>) and the sample (A<sub>Sample</sub>) from the display.
- Tap the <START> button to start the measurement procedure for the next sample.

### 6.1.5 Analytical Quality Assurance

- The method can be checked using Phosphate standard solution traceable to SRM from NIST KH<sub>2</sub>PO<sub>4</sub> in H<sub>2</sub>O 1000 mg/L PO<sub>4</sub> Certipur® (**1.19898**), which corresponds to 747.3 mg/L P<sub>2</sub>O<sub>5</sub>. This solution is diluted to 10 mg/100 mL P<sub>2</sub>O<sub>5</sub> (= 100 mg/L P<sub>2</sub>O<sub>5</sub>) with water for analysis or distilled water. To do this, place 2.676 mL **Cat. No. 1.19898** Phosphate standard solution 1000 mg/L PO<sub>4</sub> into a 20 mL volumetric flask and fill up to the mark with distilled water.
- Mix 2 mL of the prepared Standard solution with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.
- Measure this solution versus a reagent blank as described in the section "Measurement". Hereby enter a weight of 1.00 g.

**Note:** Due to the different sample preparation procedure and phosphorus determination procedure of the 10 mg/100 mL P<sub>2</sub>O<sub>5</sub> standard solution compared to a sample analysis it is necessary to recalculate the displayed result manually as follows:

$$\text{Measured Concentration standard} \left[ \frac{\text{mg}}{100 \text{ mL}} \right] = \text{Displayed result [g/100 g]} \cdot \frac{F_1}{F_2} = \text{Displayed result [g/100 g]} \cdot \frac{1000}{100}$$

$$\text{Measured Concentration standard} \left[ \frac{\text{mg}}{100 \text{ mL}} \right] = \text{Displayed result [g/100 g]} \cdot 10$$

Where, F<sub>1</sub> = 1000 = recalculation g/100 g to mg/100 mL  
F<sub>2</sub> = 100 = Factor sample preparation for real sample



## Adjustment

- In case of significant deviations in the method control procedure the preprogrammed factor of 27.99 or the current factor used in the calculation of the displayed results can be adjusted by the user.
- The corrected factor must be recalculated as follows:  
**Factor corrected = Current factor x (target value standard / measured and recalculated value standard)**
- To edit the preprogrammed factor, select method 2533 from <Methods>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list “FACTORS”.
- Tip on the input field “Factor”, enter the corrected factor and confirm by clicking on <OK>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- For the next measurement restart the method by selecting the method anew from <Methods>.

### Note:

- To find the used factor, select Method 2533 from <Methods>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list “FACTORS”.

## 6.2 Phosphorus (Total) in Meat and Sausage Products

### 6.2.1 Method

Most foods containing phosphorus compounds. Especially foods with rich content of proteins like milk and dairy products or meat and poultry are sources of phosphorus compounds.

After combustion of the sample orthophosphate ions react in sulfuric acid with react with molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) that is determined photometrically.

### 6.2.2 Measuring Range

Spectroquant® Phosphate Cell Test (1.14543):	Test kit	0.05–5.00 mg/L P
	Method	0.05–5.00 g/kg P
Spectroquant® Phosphate Cell Test (1.14729):	Test kit	0.5–25.0 mg/L P
	Method	0.5–25.0 g/kg P
Spectroquant® Phosphate Test (1.14848):	Test kit	0.005–5.00 mg/L P
	Method	0.005–5.00 mg/kg P

### 6.2.3 Reagents, Instruments and Materials

#### Reagents & Test Kits

- Spectroquant® Phosphate Cell Test (1.14543)
- Spectroquant® Phosphate Cell Test (1.14729)
- Spectroquant® Phosphate Test (1.14848)
- Sodium carbonate water-free for analysis (1.06392)
- Hydrochloric acid 37% for analysis (1.00317)
- Nitric acid 65% for analysis (1.00452)
- Water for analysis, EMSURE® (1.16754)

## Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)
- Spectroquant® Colorimeter Move 100 (**1.73632**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Nova and Prove series.

## Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

## Instrument Accessories

- Rectangular cells 10 mm (**1.14946**) or
- Rectangular cells 20 mm (**1.14947**) or
- Rectangular cells 50 mm (**1.14944**) or
- Semi-microcells 50 mm (**1.73502**)

**Note:** Rectangular cells are only necessary if the Spectroquant® Phosphate test **1.14848** is used.

## Other Reagents and Accessories

- MQuant® Phosphate Test (**1.10428**)
- Hydrochloric acid 25% for analysis EMSURE® (**1.00316**)
- Analytical balance
- Crucible
- Drying-kiln
- muffle furnace
- Heating plate
- Glass beaker, 400 mL
- Volumetric flask, 250 and 500 mL
- Volumetric pipette, 25 mL
- Folded filters

## 6.2.4 Analytical Procedure

### Sample Preparation

In a crucible mix exactly 5 g of the fine cut sample with 2 g of water-free sodium carbonate. In the drying-kiln pre-dry the content for 30 minutes at 120–130 °C and then decompose it in the muffle at 500 °C for another 30 minutes. After cooling, dissolve the residue with some water for analysis and transfer by rinsing it with water for analysis into a 400 mL glass beaker. Then slowly add 15 mL of hydrochloric acid 37% and 2 mL of nitric acid 65%. Evaporate the mixture to about 10 mL. Transfer by rinsing the residue with water for analysis into a 500 mL standard volumetric flask and fill up to volume with water for analysis. Then mix well and filter off the insoluble parts. Pipette 25 mL off the filtrate, put it into a 250 mL standard volumetric flask, fill up to volume with water for analysis and mix well (=pretreated sample).

### Using Cat. No. 1.14543: Procedure and Measurement

For more information on the measurement see the packaging insert of the test.

## Procedure

- Pipette 5.0 mL pretreated sample into a reaction cell and mix.
- Add 5 drops reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake **vigorously until the reagent is completely dissolved.**
- **Leave to stand for 5 min (reaction time),** then measure the sample in the photometer.

## Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

## Using Cat. No. 1.14729: Procedure and Measurement

For more information on the measurement see the packaging insert of the test.

## Procedure

- Pipette 1.0 mL pretreated sample into a reaction cell and mix.
- Add 5 drops reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake **vigorously until the reagent is completely dissolved.**
- **Leave to stand for 5 min (reaction time),** then measure the sample in the photometer.

## Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16-mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

## Using Cat. No. 1.14848: Procedure and Measurement

For more information on the measurement see the packaging insert of the test.

## Procedure

- Pipette 5.0 mL pretreated sample into a test tube.
- Add 5 drops reagent PO<sub>4</sub>-1 and mix.
- Add 1 level blue microspoon (in the cap of the PO<sub>4</sub>-2 bottle) reagent PO<sub>4</sub>-2 and shake **vigorously until the reagent is completely dissolved.**
- **Leave to stand for 5 min (reaction time)**, then fill the sample into the cell, and measure the sample in the photometer.

**Note:** For measurement in the **50 mm cell** both the sample volume as well as the quantity of reagents PO<sub>4</sub>-1 and PO<sub>4</sub>-2 must be **doubled**. Alternatively, the semi-microcell **Cat. No. 1.73502** can be used. It is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.

## Measurement

It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.

- If a 50 mm cell is used, perform the reagent blank one time for each measurement series. To do this tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.
- After the reagent blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

**Hint:** The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a Nova 60 A or a Move 100 is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

### 6.2.5 Calculation

Phosphorus (total) content in mg/kg P = analysis value in mg/L P x 1000

### 6.2.6 Analytical Quality Assurance

Analytical Quality Assurance (AQA) is recommended before each measurement series.

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the ortho-phosphate standard solutions (see section 5 of the respective test instruction) or Spectroquant® CombiCheck 10 or Spectroquant® CombiCheck 20 and 80, respectively can be used. Besides a standard solution with 0.80 mg/L PO<sub>4</sub>-P (or 8.0 mg/L PO<sub>4</sub>-P or 15.0 mg/L PO<sub>4</sub>-P, respectively), CombiCheck 10, 20 and 80 also contain an addition solution for determining sample-dependent interferences (matrix effects).

To view additional notes, visit **[SigmaAldrich.com/qa-test-kits](https://www.sigmaaldrich.com/qa-test-kits)**.

## 7. pH Value

The measurement of the pH in meat plays a central role in the processing of meat products and the assessment of their quality. The slaughtering procedure triggers a series of biochemical processes that result in a shift of the initial value (pH 7.0) into the acidic range (pH 5.4–6.7). The temporal course of the drop in pH depends on a variety of factors, such as the body temperature of the animals being slaughtered or the degree of stress to which the animals are subjected prior to slaughtering. A substantial percentage of the muscles of pigs, but also of those of cattle, exhibit an abnormal pattern e.g. in the rapidly glycolyzing muscle tissue.

The pH can only be measured swiftly and accurately using conventional glass or puncture electrodes under the provision that specific conditions are observed. Inadequate training in the proper use of the pH-meter frequently results in errors and false measurement results. What's more, glass electrodes are highly fragile and relatively expensive.

The pH can also be measured using color indicators, based on the principle that their color changes when a certain pH is reached. An appropriate combination of such mixed indicators can be used to achieve a good degree of accuracy. We have used this combined approach to develop a special indicator test strip for the measurement of pH levels in meat. Furthermore, the dye that is used is bound in such a way that it is made insoluble and cannot "bleed", meaning that the meat under investigation is not contaminated by the indicator dyes.<sup>18</sup>

### 7.1 pH Measurement in Meat

#### 7.1.1 Method

A special non-bleeding combination strip (**1.09632**) has been created for the pH range of 5.2–7.2 to enable the pH to be measured directly in meat with an accuracy that compares favorably with the electrometric method.

#### 7.1.2 Measuring Range

pH 5.2–7.2

#### 7.1.3 Reagents, Instruments and Materials

##### Test/Reagents Kit

- MQuant® pH indicator strips pH 5.2–7.2 for pH measurements in meat (**1.09632**)

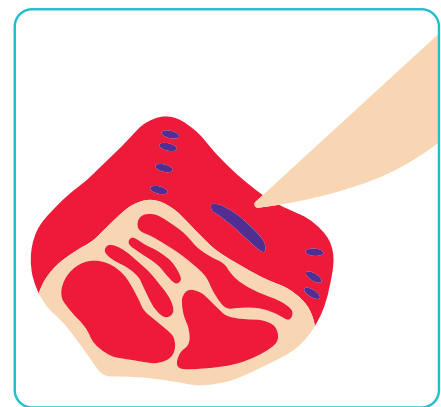
#### 7.1.4 Analytical Procedure

##### Sample Preparation

pH values can vary considerably from one region of muscles in the slaughtered animal to the next.

For this reason, it is advisable to first define a specific site in the body of the animal suitable for serial measurements, e.g. in the topside. The pH can be measured not only in the carcass, but also in boned cuts, using the special indicator test strip to determine the quality of the meat. Here it is particularly suited for identifying all DFD or "dark cutting" muscles. To ensure that a secure and reproducible reaction is achieved here, the following procedure is advisable:

- The chosen site must be undamaged (free from blood, lacerations etc.).
- Using a sharp-tipped knife, make an incision across the direction of the muscle fibers (about one inch deep).

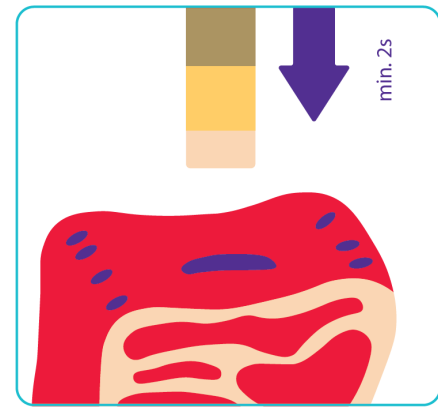


Sample preparation

## Measurement

Carefully insert the special indicator test strip into the incision to a depth of about 3/4 inch, or 20 mm, and press the muscle together above and below the measurement site for 2–5 seconds. This is to ensure good contact of the muscle with the indicator zones and that they are thoroughly wetted with the meat juices.

When carrying out this procedure, it must be borne in mind that the indicator zones are relatively sensitive to moisture and should not be touched with the bare fingers.



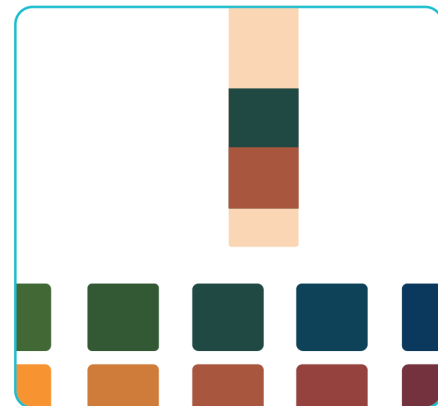
Measurement of pH in meat

## Color Comparison

The color comparison scale has the following color graduations:

5.2–5.6–6.0–6.4–6.8–7.2

Remove the test strip from the incision and, under adequate lighting conditions, compare the two reaction zones with the color scale printed on the pack until the best possible color match is achieved. Read off the corresponding pH value.



Color comparison

## 7.1.5 Conclusion

### Five advantages of the special indicator for measuring pH in meat

The advantages of this specially developed pH indicator test strip (**1.09632**) for pH measurement in meat at a glance:

- No “contamination” of the measurement area thanks to the covalent binding of the indicator dyes.
- The test-strip principle enables the measurement to be made directly in the meat (the plastic material is physiologically safe).
- No protein error.
- No impact of the inherent color of the meat or meat juices.
- Improved color graduation makes it easy to read off the pH results visually.



## 8. Ordering Information

### Featured Products

Description	Cat. No.
<b>Reagent kits, Test Kits and Test Strips</b>	
Spectroquant® Nitrite Cell Test, 0.03–2.30 mg/L NO <sub>2</sub> <sup>-</sup>	1.14547
Spectroquant® Nitrite Test, 0.007–3.28 mg/L NO <sub>2</sub> <sup>-</sup>	1.14776
Reflectoquant® Nitrite Test, 0.5–25.0 mg/L NO <sub>2</sub> <sup>-</sup>	1.16973
Spectroquant® Chloride Cell Test, 5–125 mg/L Cl <sup>-</sup>	1.14730
Spectroquant® Chloride Test, 2.5–250 mg/L Cl <sup>-</sup>	1.14897
Spectroquant® Phosphate Cell Test, 0.2–15.3 mg/L PO <sub>4</sub> <sup>3-</sup>	1.14543
Spectroquant® Phosphate Cell Test, 1.5–76.7 mg/L PO <sub>4</sub> <sup>3-</sup>	1.14729
Spectroquant® Phosphate Test, 0.015–15.3 mg/L PO <sub>4</sub> <sup>3-</sup>	1.14848
MQuant® pH indicator strips pH 5.2–7.2 for pH measurements in meat	1.09632
<b>Instruments and Accessories</b>	
Spectroquant® VIS Spectrophotometer Prove 100 plus	1.73026
Reflectometer RQflex® 20, pkg of 1 unit, Reflectoquant®	1.17246
RQcheck Set for RQflex® 20 Reflectometer, 1 set	1.17247
Recalibration Set for RQflex® 20 Reflectometer, 1 unit	1.16954
Rectangular cells 10 mm	1.14946
Rectangular cells 20 mm	1.14947
Rectangular cells 50 mm	1.14944
<b>Reagents</b>	
Potassium hydrogen phthalate for analysis EMSURE® Reag. Ph Eur	1.04874
MQuant® pH-indicator strips pH 0–6.0	1.09351
Carrez Clarification Kit reagent kit for sample preparation in food analysis, 5-fold	1.10537
Sodium hydroxide solution c(NaOH) = 1 mol/L (1 N) Titripur® Reag. Ph Eur, Reag. USP	1.09137
MQuant® Nitrite Test, 2–80 mg/L NO <sub>2</sub> <sup>-</sup>	1.10007
Nitrite standard solution Certipur®, 1000 mg/L NO <sub>2</sub> <sup>-</sup>	1.19899
Water for analysis EMSURE®	1.16754
Chloramine T Trihydrate for analysis	1.02426
Hydrochloric acid solution 6.0 mol/L (6.0 N)	1.43007
Petroleum benzine boiling range 40–60 °C SupraSolv®	1.01722
Citric acid monohydrate for analysis EMSURE®	1.00244
Sodium hydroxide pellets for analysis EMSURE®	1.06498
Sodium acetate anhydrous for analysis EMSURE®	1.06268
Perchloric acid 60% for analysis EMSURE®	1.00518
1-Propanol for analysis EMSURE®	1.00997
2-Propanol for analysis EMSURE®	1.09634
Nitric acid 65% for analysis EMSURE®	1.00452
Ammonium monovanadate GR for analysis	1.01226
Ammonium heptamolybdate tetrahydrate GR for analysis	1.01182
Phosphate standard solution traceable to SRM from NIST KH <sub>2</sub> PO <sub>4</sub> in H <sub>2</sub> O 1000 mg/L PO <sub>4</sub> Certipur®	1.19898
Hydrochloric acid 37% for analysis	1.00317
Sodium carbonate water-free for analysis	1.06392

## Related Products

Description	Cat. No.
<b>Instruments and Accessories</b>	
Spectroquant® UV/VIS Spectrophotometer Prove 300 plus	1.73027
Spectroquant® UV/VIS Spectrophotometer Prove 600 plus	1.73028
Spectroquant® colorimeter Move 100	1.73632
Semi-microcells 50 mm	1.73502
<b>Reagents</b>	
Nitrite standard solution, 0.200 mg/L NO <sub>2</sub> -N	1.25041
Sulfuric acid 0.5 mol/L Titripur®	1.09072
Spectroquant® CombiCheck 10	1.14676
Spectroquant® CombiCheck 20	1.14675
Spectroquant® CombiCheck 50	1.14696
Spectroquant® Chloride standard solution, 10.0 mg/L Cl	1.32229
Spectroquant® Chloride standard solution, 50 mg/L Cl	1.32230
MQuant® Phosphate Test, 10–500 mg/L PO <sub>4</sub> <sup>3-</sup>	1.10428
Hydrochloric acid 25% for analysis EMSURE®	1.00316

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