

Reducing Sugar Content Assay Kit (Fehling's Titration Method)

Cat. No.: R151034 | Pack size: 40T | Storage: Store at 2-8 °C, Room temperature

Overview

Fehling's Reagent (also known as Fehling's solution) was invented by the German chemist Hermann von Fehling in 1849. Similar to Benedict's Reagent, it is used to detect the presence of reducing sugars. The principle is that soluble reducing sugars (such as glucose, fructose, and maltose) react with the reagent under heating conditions to form a brick-red precipitate of cuprous oxide (Cu_2O).

This kit is based on the method (direct titration) recommended by the National Standard of China (GB/T 5009.7-2016 – Determination of Reducing Sugars in Foods). It is mainly composed of potassium sodium tartrate, copper sulfate, methylene blue, potassium ferrocyanide, glucose standard, etc. It is primarily used for the quantitative determination of reducing sugars in foods such as starch-containing foods, alcoholic beverages, carbonated drinks, meat products, and preserved fruits. Total sugar content can also be measured but requires prior hydrolysis. It can also be used for qualitative tests of reducing sugars.

The detection principle of this method is as follows: after deproteinization of the sample, using methylene blue as an indicator, a standardized Fehling's reagent (equal volumes of Solution A and B are mixed to form a soluble blue complex of potassium sodium tartrate-copper, also called alkaline copper tartrate solution) is titrated under heating conditions. The reducing sugars in the sample reduce the divalent copper in the potassium sodium tartrate-copper complex to red cuprous oxide precipitate. The cuprous oxide precipitate then reacts with potassium ferrocyanide to form a soluble colorless complex. When all divalent copper is reduced, a slight excess of reducing sugar reduces the methylene blue, changing the solution from blue to colorless, which is the titration endpoint. The reducing sugar content is calculated based on the volume of sample solution consumed. This product is not pre-standardized; users must standardize it themselves. This kit is for research use only and is not suitable for clinical diagnosis or other purposes.

Contents & Storage

Cat. No.	Component	40T	Storage
R151034A	Glucose Standard (1 mg/mL)	50 mL	2-8 °C
R151034B	Fehling's Reagent A	250 mL	RT

Cat. No.	Component	40T	Storage
R151034C	Fehling's Reagent B	250 mL	RT
R151034D	Zinc Acetate Solution	200 mL	RT
R151034E	Potassium Ferrocyanide Solution	200 mL	RT
R151034F	Alkaline Solution	50 mL	RT

Grade / purity: BioReagent.

Reagents, consumables and Equipments not provided

Test tubes, conical flasks, volumetric flasks, glass beads, water bath, alcohol burner or other heating device, analytical balance, acid burette.

Reducing sugar standards (1 mg/mL) such as fructose or invert sugar.

Operating Steps (For Reference Only)

1. Standardization of Fehling's Reagent

Fill an acid burette (25 mL) with about 15 mL of reducing sugar standard solution (1 mg/mL). Into a 150 mL conical flask, add 5 mL each of Fehling's Reagent A and B, then add 10 mL of water and 2–4 glass beads. Heat the flask to boiling within 2 minutes. Maintain boiling and titrate with the reducing sugar standard solution at a rate of about 1 drop every 2 seconds until the blue color just disappears as the endpoint. Record the total volume of standard solution consumed. Perform in triplicate and take the average. Calculate the mass (mg) of reducing sugar equivalent to 10 mL of Fehling's Reagent (5 mL each of A and B).

2. Sample Preparation

2.1 Starch-containing Foods

Weigh 10–20 g (accurate to 0.001 g) of pulverized or homogenized sample into a 250 mL volumetric flask, add 200 mL of water, and heat in a 45 °C water bath for 1 hour with occasional shaking. Cool, dilute to the mark with water, mix well, and let stand. After settling, pipette 200 mL of the supernatant into another 250 mL volumetric flask. Slowly add 5 mL each of Zinc Acetate Solution and Potassium Ferrocyanide Solution. Dilute to the mark with water, mix well, and let stand for 30 minutes. Filter through dry filter paper, discard the initial filtrate, and keep the subsequent filtrate for use.

2.2 Alcoholic Beverages

Weigh 100 g (accurate to 0.01 g) of well-mixed sample into an evaporating dish. Neutralize with Alkaline Solution, then evaporate on a water bath to one-quarter of the original volume. Transfer to a 250 mL volumetric flask. Slowly add 5 mL each of Zinc Acetate Solution and Potassium Ferrocyanide Solution. Dilute to the mark with water, mix well, let stand for 30 minutes, filter through dry filter paper, discard the initial filtrate, and keep the subsequent filtrate for use.

2.3 Carbonated Beverages

Weigh 100 g (accurate to 0.01 g) of well-mixed sample into an evaporating dish. Gently stir on a water bath to remove carbon dioxide. Transfer to a 250 mL volumetric flask. Rinse the dish with water and combine the rinses into the flask. Dilute to the mark with water and mix well for use.

2.4 Other Foods

Weigh 2.5–5 g (accurate to 0.001 g) of pulverized solid sample or 5–25 g (accurate to 0.001 g) of homogenized liquid sample into a 250 mL volumetric flask. Add 50 mL of water, then slowly add 5 mL each of Zinc Acetate Solution and Potassium Ferrocyanide Solution. Dilute to the mark with water, mix well, let stand for 30 minutes, filter through dry filter paper, discard the initial filtrate, and keep the subsequent filtrate for use.

3. Preliminary Titration of Sample Solution

Fill an acid burette (25 mL) with about 15 mL of the sample solution. Into a 150 mL conical flask, add 5 mL each of Fehling's Reagent A and B, then add 10 mL of water and 2–4 glass beads. Heat the flask to boiling within 2 minutes. Maintain boiling and titrate with the sample solution, starting quickly and then slowing down. When the color becomes lighter, titrate at about 1 drop every 2 seconds until the blue color just disappears as the endpoint. Record the total volume of sample solution consumed. (Note: If the reducing sugar concentration in the sample solution is too high, dilute appropriately before measurement. Try to make the volume of sample solution consumed in each titration similar to the volume of reducing sugar standard solution consumed during standardization, approximately 10 mL. When the concentration is too low, directly add 10 mL of sample solution (without the extra 10 mL water) and then titrate with the reducing sugar standard solution to the endpoint. The difference between the volume consumed and the volume consumed during standardization corresponds to the mass of reducing sugar in 10 mL of sample solution.)

4. Formal Titration of Sample Solution

Fill an acid burette (25 mL) with about 20 mL of the sample solution. Into a 150 mL conical flask, add 5 mL each of Fehling's Reagent A and B, then add 10 mL of water and 2–4 glass beads. Heat the flask to boiling within 2 minutes. Maintain boiling and titrate with the sample solution at a rate of about 1 drop every 2 seconds until the blue color just disappears as the endpoint. Record the total volume of sample solution consumed. Perform in triplicate and take the average.

5. Result Calculation

The reducing sugar content in the sample (expressed as a specific reducing sugar) is calculated using the formula below:

$$X = m_1 \times 100 / (m \times F \times V / 250 \times 1000)$$

When the concentration is too low, use the following formula

$$X = m_2 \times 100 / (m \times F \times 10 / 250 \times 1000)$$

Parameter Explanation

X: Reducing sugar content in the sample (expressed as a specific reducing sugar) (g/100 g).

m_1 : Mass (mg) of a specific reducing sugar equivalent to Fehling's Reagent (half each of A and B).

m_2 : Mass (mg) of a specific reducing sugar corresponding to the difference between the volume consumed during standardization and the volume of reducing sugar standard solution consumed after adding the sample.

m: Sample mass (g).

F: Sample coefficient: 0.8 for starch-containing foods, 1 for others.

V: Average volume of sample solution consumed during measurement (mL).

10: Volume of sample solution (mL) used in the low-concentration method.

250: Volumetric flask volume (mL).

1000: Unit conversion factor.

Reporting of results

When reducing sugar content ≥ 10 g/100 g, report with three significant figures.

When reducing sugar content < 10 g/100 g, report with two significant figures.

Appendix 1: Qualitative Test for Reducing Sugars

1. Prepare Fehling's Reagent Working Solution: Immediately before use, mix equal volumes of Fehling's Reagent A and B.
2. Add 1–2 mL of the test sample to a clean test tube.
3. Add 1 mL of the Fehling's Reagent working solution to the tube and mix thoroughly.
4. Place the mixture in a boiling water bath for 1–3 minutes.
5. Observe the color change of the mixture. The color should change in the order: light blue $\rightarrow$ brown $\rightarrow$ brick red (precipitate).

Appendix 2: Hydrolysis and Extraction of Total Sugars

1. Weigh 0.5–3 g of plant sample, cut into pieces, add about 3 mL of distilled water, and homogenize. Transfer to a beaker or conical flask. Rinse the homogenizer 2–3 times with 12 mL of distilled water and combine the rinses into the container.
2. Add 10 mL of 6 M hydrochloric acid solution to the container, stir well, and boil for 30 minutes with occasional stirring.
3. Place 2 drops of the hydrolysate on a glass slide and add 1 drop (about 50 μ L) of chromogenic solution to check for complete hydrolysis. No blue color indicates complete hydrolysis.
4. After hydrolysis, cool to room temperature. Add 6 M sodium hydroxide solution to adjust the pH to 7.4. Dilute to 100 mL with distilled water, mix well, and centrifuge at 4000 g for 5 minutes or filter.
5. Take 10 mL of the supernatant or filtrate and dilute to 100 mL with distilled water to obtain a 10-fold diluted total sugar hydrolysate (extract). Take 0.5 mL of this total sugar hydrolysate to measure its reducing sugar content.

Interpretation of Results

Sample Type	Observation
Reducing Sugar (e.g., ribose, glucose, fructose)	Brick red precipitate
Non-reducing Sugar (e.g., sucrose, starch)	No color change

Precautions

1. To measure total sugar content, hydrolyze the sample to reducing sugars first and then refer to the method for reducing sugar determination.
2. If the reducing sugar concentration in the sample solution is too high, dilute appropriately before measurement. Try to keep the volume of sample solution consumed in each titration similar to the volume of reducing sugar standard solution consumed during standardization.
3. When the reducing sugar concentration in the sample solution is too low, directly add 10 mL of sample solution (without the extra 10 mL water) and titrate with the reducing sugar standard solution to the endpoint. The difference between the volume consumed and the volume consumed during standardization corresponds to the mass of reducing sugar in 10 mL of sample solution.
4. You may also standardize 4–20 mL of Fehling's Reagent (half each of A and B) according to the above method to adapt to variations in reducing sugar concentration in samples.

5. Fehling's Reagent A and B must be stored separately and mixed in proportion immediately before use.
6. Fehling's Reagent B is strongly alkaline; handle with care. It contains potassium ferrocyanide, which eliminates interference from cuprous oxide precipitate at the titration endpoint.
7. This kit is suitable for the determination of reducing sugars in various foods. However, when analyzing dark-colored samples such as soy sauce, pigments may interfere, making the titration endpoint unclear and affecting accuracy. Decolorize before measurement. Decolorization methods and amounts of decolorizing agents vary for different samples; please consult relevant literature. For example, 5% activated charcoal can be used for decolorizing red wine.
8. Please use the reagent as soon as possible after opening to avoid affecting subsequent experimental results.

Specifications

Attribute	Value
Storage	Store at 2-8 °C, Room temperature
Shipped In	Wet ice
Stability and Storage	Each component has a shelf life of 1 year under corresponding storage conditions.
Specifications & Purity	BioReagent

Quality Control

QC Item	Method	Acceptable Range
Documentation review	Lot-specific COA and product label review	Catalog number, lot number, pack size, storage condition, and release status are consistent with approved records.
Appearance / integrity	Visual inspection on receipt	Container is intact; reagent appearance conforms to lot-specific specifications.
Functional suitability	Application-specific internal QC or reference procedure	Performance meets the approved lot-specific acceptance criteria.

Troubleshooting

Issue	Possible Causes	Corrective Action
Weak or no expected signal / staining / reaction	• Reagent not equilibrated or stored correctly • Insufficient incubation or reaction time • Sample condition outside recommended range	• Verify storage conditions and expiration status • Optimize incubation/reaction time using a positive control • Use fresh or appropriately prepared samples
High background or nonspecific result	• Over-staining or excessive reaction time • Incomplete washing, differentiation, or cleanup • Sample matrix interference	• Shorten staining/reaction time or dilute reagent if applicable • Increase washing/differentiation stringency • Include blank and negative controls
Inconsistent replicate results	• Uneven sample preparation or mixing • Pipetting variability • Temperature or timing variation	• Standardize sample preparation and mixing • Use calibrated pipettes and consistent timing • Run controls with each batch

Recommended Applications

Research use associated with Reducing Sugar Content Assay Kit (Fehling's Titration Method).

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