

Product Information

TOTAL DIETARY FIBER ASSAY KIT

Product Codes **TDF-100A** and **TDF-C10**

TECHNICAL BULLETIN

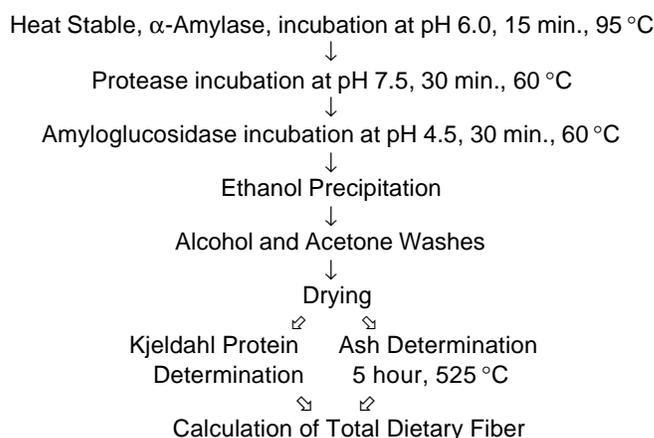
Introduction

Dietary fiber is a mixture of complex organic substances and was initially defined as remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man.¹ This definition was modified to include hemicelluloses, celluloses, lignins, pectins, gums, nondigestible oligosaccharides, and waxes.^{2,3} This broader definition acknowledges the significance of fiber as a chemical and physiological component of the diet. This procedure for the determination of total dietary fiber is based on the method published in the 16th Edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).⁴

Summary of Procedure

This assay determines the total dietary fiber content of food using a combination of enzymatic and gravimetric methods. Samples of dried, fat-free foods are gelatinized with heat stable α -amylase and then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present in the sample. Ethanol is added to precipitate the soluble dietary fiber. The residue is then filtered and washed with ethanol and acetone. After drying, the residue is weighed. Half of the samples are analyzed for protein and the others are ashed. Total dietary fiber is the weight of the residue less the weight of the protein and ash.

TOTAL DIETARY FIBER ASSAY



Reagents Provided:

TDF-100A Kit – Total Dietary Fiber Assay Kit. This kit contains enough reagents to perform 100 assays.

1. α -Amylase, Heat Stable; Product Code A3306
2. Protease; Product Code P3910
3. Amyloglucosidase; Product Code A9913
4. Celite™, Acid Washed; Product Code C8656

TDF-C10 Kit – Total Dietary Fiber Assay Control Kit, Each bottle contains enough material for approximately 10 assays.

1. Arabinogalactan; Product Code A9788
2. Casein; Product Code C7906
3. β -Glucan; Product Code G7391
4. Pectin; Product Code P7536
5. Starch, Corn; Product Code S2388
6. Starch, Wheat; Product Code S1514

Reagents Required but Not Provided;

1. Petroleum ether; Product Code 18,451-9
2. Ethyl Alcohol, ACS reagent; Product Code 45,984-4
3. Acetone, ACS reagent; Product Code 32,011-0
4. Sodium Phosphate, Dibasic, anhydrous; Product Code S0876
5. Sodium Phosphate, Monobasic, anhydrous; Product Code S0751
6. Sodium Hydroxide, 1.0 N; Product Code 930-65
7. Hydrochloric Acid, 1.0 M HCl; Product Code 920-1

Apparatus

1. Fritted crucible-porosity #2 (coarse 40-60 microns)
2. Vacuum source. A vacuum pump or aspirator equipped with an inline double vacuum flask should be used to prevent contamination in case of water backup.
3. An air oven capable of operating at 105 °C or a vacuum oven set at 70 °C.
4. Desiccator
5. Muffle furnace
6. Boiling water bath
7. Constant temperature water bath adjustable to 60 °C with either a multistation shaker or multistation magnetic stirrer to provide agitation of the digestion flasks during enzymatic hydrolysis.
8. Beakers - 400 ml or 600 ml tall form
9. Analytical balance capable of weighing to 0.1 mg.
10. pH meter - standardized at pH 4.0 and pH 7.0.

Preparation Instructions

Crucibles

Wash crucibles thoroughly. Heat one hour at 525 °C and cool. Soak and rinse crucibles in water and then air dry. Add 0.5 grams of Celite to each crucible and dry at 130 °C to constant weight (one hour or more). Cool in desiccator and weigh to nearest 0.1 mg. Record this as "Celite + Crucible Weight" or W_1 . Store in desiccator until needed.

Sample

If the fat content of the sample is greater than 10%, defat with petroleum ether.⁴

Record the loss of weight due to fat removal and make the appropriate correction to the final % dietary fiber. When dealing with unknowns, fat-extract all samples.

Homogenize each sample, if necessary, and dry overnight in an air oven at 105 °C (70 °C in vacuum oven). Cool in desiccator and dry mill sample to 0.3-0.5 mm mesh. If apparatus is unavailable for milling, grinding in a mortar will be sufficient. If sample cannot be heated, freeze dry before milling. Store dry sample in a desiccator until analysis is carried out.

Reagents

Use distilled or deionized water to prepare solutions.

1. 78% Ethanol

Place 207 ml of water into a one liter volumetric flask. Dilute to volume with 95% ethanol. Mix and bring to volume again with 95% ethanol if necessary. Mix.

2. Phosphate Buffer, 0.08 M, pH 6.0

Dissolve 1.4 g of Na_2HPO_4 (Product Code S 0876) and 8.4 g of NaH_2PO_4 , anhydrous (Product Code S 0751) in approximately 700 ml of water. Dilute to one liter with water. Check pH and adjust if necessary with either NaOH or H_3PO_4 . Store in tightly capped container at room temperature.

3. Sodium Hydroxide Solution, 0.275 N

Dilute 275 ml of 1.0 N NaOH solution (Product Code 930-65) to one liter with water in a volumetric flask. Store in a tightly capped container at room temperature.

4. Hydrochloric Acid Solution, 0.325 M

Dilute 325 ml of 1.0 M HCl solution (Product Code 920-1) to one liter with water in a volumetric flask. Store in a tightly capped container at room temperature.

Determination

Run blanks along with samples through the entire procedure to measure any contributions to residue from reagents. Samples and blanks to be tested for dietary fiber content should be run at least in quadruplicate so that duplicate protein and ash values are available for improved accuracy.

1. Weigh out four 1-gram samples of each material to be tested into tall form beakers. Sample weights should not differ by more than 20 mg. Record weights to 0.1 mg.
2. Add 50 ml of pH 6.0 phosphate buffer to each beaker.
3. Add 0.10 ml α -Amylase (Product Code A3306) to each beaker and mix well.
4. Cover each beaker with aluminum foil and place in a boiling water bath. Agitate beakers gently at 5 minute intervals. Incubate for 15 minutes after the internal temperature of the beakers reaches 95 °C.
5. Allow solutions to cool to room temperature.
6. Adjust the pH of the solutions to 7.5 ± 0.2 by adding 10 ml of 0.275 N NaOH to each beaker. Check pH, adjust if necessary with either NaOH or HCl.
7. Immediately before use, make a 50 mg/ml solution of Protease (Product Code P3910) in phosphate buffer. Pipette 0.1 ml (5 mg Protease) into each beaker.
8. Cover each beaker with aluminum foil and place in 60 °C water bath. With continuous agitation, incubate for 30 minutes after the internal temperature of the beakers reaches 60 °C.
9. Allow solutions to cool to room temperature.
10. Adjust the pH of the solutions to between pH 4.0 and 4.6 by adding 10 ml of 0.325 M HCl to each beaker. Check pH, adjust if necessary with either NaOH or HCl.
11. Add 0.1 ml of Amyloglucosidase (Product Code A9913) to each beaker.
12. Cover each beaker with aluminum foil and place in 60 °C water bath. With continuous agitation, incubate for 30 minutes after the internal temperature of the beakers reaches 60 °C.
13. Add 4 volumes of 95% ethanol to each beaker.
14. Let solutions set overnight at room temperature to allow complete precipitation.
15. Filtration

Wet and redistribute the bed of Celite in each crucible using 78% ethanol. Apply gentle suction to draw Celite onto frit as an even mat. Maintain gentle suction and quantitatively transfer the precipitate and suspension from each beaker to its respective crucible.

Wash the residue with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol, and two 10 ml portions of acetone.

A gum may form with some samples, trapping liquid. Breaking the surface film with a spatula will improve the rate of filtration. Be sure to rinse any material adhering to the spatula into the crucible. The time for filtration and washing will vary from 0.1 to 6 hours per crucible, averaging about 0.5 hour per crucible.
16. Dry crucibles containing residues overnight in a 105 °C air oven or 70 °C vacuum oven.
17. Cool all crucibles in a desiccator, weigh to nearest 0.1 mg, and record this weight as "Residue + Celite + Crucible Weight" or W_2 .
18. Analyze the residues from two samples and two blanks for protein by Kjeldahl nitrogen analysis as specified in the AOAC procedure.⁵ Use 6.25 as the factor to convert ammonia determined in the analysis to protein except where nitrogen content in the protein sample is known.
19. Ash the residue in the crucibles from two samples and two blanks for 5 hours at 525 °C. Cool in desiccator, weigh to nearest 0.1 mg and record this weight as "Ash + Celite + Crucible Weight" or W_3 .

Enzyme Effectiveness Testing

The enzymes included in the TDF-100A Kit have been evaluated using the test samples listed in the table below. Full enzymatic activity and the absence of undesirable contaminating activities is indicated by obtaining the expected recovery of dietary fiber for each test sample. It is advisable to include Casein (Product Code C7906) and/or Corn Starch (Product Code

S2388) in each determination as internal controls. About every six months after the kit enzymes have been opened, each of the test samples listed below should be run through the entire assay for total dietary fiber to ensure that no degradation or contamination of the enzymes has occurred.

Test Sample	Sigma Product Code	Activity	Sample Weight	Expected Recovery % Dietary Fiber
Arabinogalactan	A9788	Hemicellulase*	0.1 gram	95-100
Casein	C7906	Protease**	0.3 gram	0-2
β-Glucan	G7391	β-Glucanase*	0.1 gram	95-100
Pectin	P7536	Pectinase*	0.1 gram	95-100
Starch, Corn	S2388	Amylase** Amyloglucosidase**	1.0 gram	0-2
Starch, Wheat	S1514	Amylase** Amyloglucosidase**	1.0 gram	0-1

* This activity should not be present in the tests.

** This activity should be fully functional in the tests.

Calculations

$$\text{Residue Weight} = W_2 - W_1 \quad \text{Ash Weight} = W_3 - W_1$$

$$B = R_{\text{BLANK}} - P_{\text{BLANK}} - A_{\text{BLANK}}$$

$$\% \text{ TDF} = [(R_{\text{SAMPLE}} - P_{\text{SAMPLE}} - A_{\text{SAMPLE}} - B) / \text{SW}] \times 100$$

Where : TDF = Total Dietary Fiber
 R = Average Residue Weight (mg)
 P = Average Protein Weight (mg)
 A = Average Ash Weight (mg)
 SW = Average Sample Weight (mg)

References

1. Trowell, H., Lancet, **1**, 504 (1974).
2. Trowell, H., et.al., Lancet, **1**, 967 (1976).
3. Van Soest, P.J. and McQueen, R.W., Proc. Nutr. Soc., **32**, 123-130 (1973).
4. Official Methods of Analysis of AOAC International, 16th Edition, Volume II, Section 45.4.07, Method 985.29 (1997).
5. Official Methods of Analysis of AOAC International, 16th Edition, Volume I, Section 12.1.07, Method 960.52 (1997).

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