Megazyme

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L-FUCOSE

ASSAY PROCEDURE

K-FUCOSE 05/20

(*100 Manual Assays per Kit) or
(1020 Auto-Analyser Assays per Kit) or
(1000 Microplate Assays per Kit)

** The number of tests per kit can be doubled if all volumes are halved*

INTRODUCTION:

L-Fucose is a 6-carbon deoxyhexose and exists in nature in various biological niches. A major natural source of L-fucose is the brown algal polysaccharide fucoidan.

L-Fucose is a minor component in plant cell wall polysaccharides, specifically rhamnogalacturonan, xyloglucan and also arabinogalactan proteins that are involved in plant cell elongation.

In glycoproteins L-fucose is a key component of the oligosaccharide moiety and is attached via α-1,(2, 3, 4 or 6) linkages. The variation of the oligosaccharide structure is known to dictate biological function of glycoproteins and, as such, L-fucose has been indicated as a potential biological marker for various disease states such as breast cancer. L-fucose is a common component of human milk oligosaccharides (HMO) such as 2'-fucosyl-lactose. In addition to the role as a prebiotic, HMOs are known to impart immunological functions to breast-fed infants such as preventing microbial adhesion to the intestinal mucosal membrane.

This kit (**K-FUCOSE**) provides a rapid and reliable method, suitable for the measurement of L-fucose in plant extracts, biological samples and other materials. It can also be used in the measurement of α-L-fucosidases that do not act on chromogenic substrates.

PRINCIPLE:

L-Fucose is oxidised by the enzyme L-fucose dehydrogenase in the presence of nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to L-fucono-1,5-lactone with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (1).

$$\text{(1) L-Fucose} + NADP^+ \xrightarrow{\text{(L-fucose dehydrogenase)}} \text{L-fucono-1,5-lactone} + NADPH + H^+$$

The amount of NADPH formed in this reaction is stoichiometric with the amount of L-fucose. It is the NADPH which is measured by the increase in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay rapidly converts L-fucose and also converts the rare monosaccharides, L-galactose and D-arabinose, but at a much reduced rate (approx. 3-fold and 50-fold slower than L-fucose, respectively). L-Arabinose, D-fucose, D-glucose, D-mannose and D-xylose do not react.

The smallest differentiating absorbance for the assay is 0.010 absorbance units. This corresponds to 0.34 mg/L of sample solution at the maximum sample volume of 2.00 mL. The detection limit is 0.68 mg/L, which is derived from an absorbance difference of 0.020

with a sample volume of 2.00 mL. The assay is linear over the range of 0.5 to 100 µg of L-fucose per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur. With a sample volume of 2.00 mL, this corresponds to an L-fucose concentration of approx. 0.17 to 0.34 mg/L of sample solution. If, in sample preparation, the sample is weighed, e.g. 10 g/L, a difference of 0.02 to 0.05 g/100 g can be expected.

INTERFERENCE:

If the conversion of L-fucose has been completed within the time specified in the assay (approx. 10 min), it can be generally concluded that no interference has occurred. However, this can be further checked by adding L-fucose (approximately 50 µg in 0.1 mL) to the cuvette on completion of the reaction. A significant increase in the absorbance should be observed.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 100 assays in manual format (or 1020 assays in auto-analyser format or 1000 assays in microplate format) are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (44 mL; pH 9.5) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: $NADP^+$.
Freeze dried powder.
Stable for > 5 years below -10°C.

Bottle 3: L-Fucose dehydrogenase suspension (5.5 mL).
Stable for > 2 years below -10°C.

Bottle 4: L-Fucose standard solution (5 mL, 0.5 mg/mL).
Stable for > 2 years; store sealed at 4°C.

PREPARATION OF REAGENT SOLUTIONS (SUPPLIED):

1. Use the contents of bottle 1 as supplied.
   Stable for > 2 years at 4°C.
2. Dissolve the contents of bottle 2 in 11 mL of distilled water. **Stable for ~ 4 weks at 4°C** or stable for > 2 years below -10°C (to avoid repetitive freeze/thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).

3. Use the contents of bottle 3 as supplied. Before opening for the first time, shake the bottle to remove any enzyme that may have settled on the rubber stopper. Subsequently store the bottle in an upright position. **Swirl the bottle to mix contents before use.**
   Stable for > 2 years below -10°C.
4. Use the contents of bottle 4 as supplied.
   Stable for > 2 years; store sealed at 4°C.

NOTE: The L-fucose standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of L-fucose is determined directly from the extinction coefficient of NADPH (see page 5).

EQUIPMENT (RECOMMENDED):

1. Volumetric flask (1 L).
2. Disposable plastic or glass cuvettes (1 cm light path, 3 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (20 µL, 200 µL and 1 mL).
4. Positive displacement pipettor, e.g. Eppendorf Multipette® - with 25 mL Combitip® (to dispense 0.4 mL aliquots of buffer 1 and 0.1 mL aliquots of $NADP^+$ solution).
5. Stop clock.
6. Spectrophotometer set at 340 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Thermostated water bath.

A. MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: ~ 37°C
Final volume: 2.65 mL
Sample solution: 0.5-100 µg of L-fucose per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
distilled water	2.10 mL	2.00 mL
sample solution	-	0.10 mL
solution 1 (buffer)	0.40 mL	0.40 mL
solution 2 ($NADP^+$)	0.10 mL	0.10 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:		
suspension 3 (L-FDH)	0.05 mL	0.05 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 10 min). If the reaction has not stopped after 10 min, continue to read the absorbances at 2 min intervals until the absorbances either plateau or decrease constantly over 2 min.		

* For example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

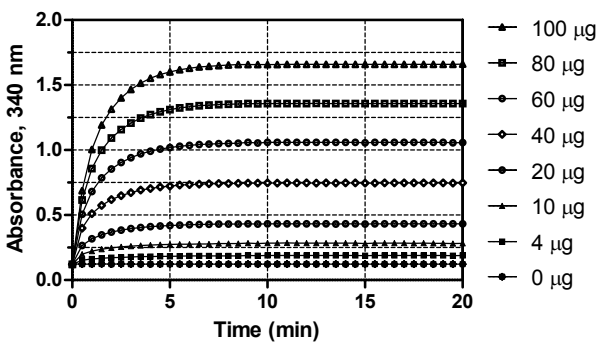

Figure 1. Increase in absorbance at 340 nm on incubation of 0-100 µg of L-fucose with L-fucose dehydrogenase in the presence of $NADP^+$ at 37°C using 1 cm path-length cuvettes (Manual Format; above).

CALCULATION (Manual Assay Procedure):

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{L\text{-fucose}}$.

The value of $\Delta A_{L\text{-fucose}}$ should be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of L-fucose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{L\text{-fucose}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of L-fucose [g/mol]
ε = extinction coefficient of NADPH at 340 nm
= 6300 [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for L-fucose:

$$c = \frac{2.65 \times 164.16}{6300 \times 1.0 \times 0.1} \times \Delta A_{L\text{-fucose}} \quad [g/L]$$

$$= 0.6905 \times \Delta A_{L\text{-fucose}} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of L-fucose:

$$= \frac{c_{L\text{-fucose}}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ sample\ solution]} \times 100 \quad [g/100\ g]$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

B. AUTO-ANALYSER ASSAY PROCEDURE:

NOTES:

1. The Auto-Analyser Assay Procedure for L-fucose can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of L-fucose **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

This kit is suitable for the preparation of 280.5 mL of reagent (equivalent to 1020 reactions of 0.275 mL). Reagent preparation is performed as follows:

Preparation of R1:

Component	Volume
distilled water solution 1 (buffer) solution 2 ($NADP^+$)	40 mL 8.8 mL 2.2 mL (after adding 11 mL of H_2O to bottle 2)
Total volume	51 mL

Preparation of R2:

Component	Volume
distilled water suspension 3 (L-FDH)	4 mL 1.1 mL
Total volume	5.1 mL

EXAMPLE METHOD:

R1:	0.250 mL
Sample:	~ 0.01 mL
R2:	0.025 mL
Reaction time:	~ 10 min at 37°C
Wavelength:	340 nm
Prepared reagent stability:	> 7 days when refrigerated
Calculation:	endpoint
Reaction direction:	increase
Linearity:	0.01-1 g/L of L-fucose using 0.01 mL sample volume

C. MICROPLATE ASSAY PROCEDURE:

NOTES:

1. The Microplate Assay Procedure for L-fucose can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of L-fucose **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 37°C
Final volume: 0.265 mL
Linearity: 0.1-10 µg of L-fucose per well (in 0.01-0.20 mL sample volume)

Pipette into wells	Blank	Sample	Standard
distilled water	0.210 mL	0.200 mL	0.200 mL
sample solution	-	0.010 mL	-
standard solution	-	-	0.010 mL
solution 1 (buffer)	0.040 mL	0.040 mL	0.040 mL
solution 2 ($NADP^+$)	0.010 mL	0.010 mL	0.010 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 4 min and start the reactions by addition of:			
suspension 3 (L-FDH)	0.005 mL	0.005 mL	0.005 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 10 min). If the reaction has not stopped after 10 min, continue to read the absorbances at 2 min intervals until the absorbances either plateau or decrease constantly over 2 min.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipettor set at 50-100 µL volume).

CALCULATION (Microplate Assay Procedure):

$$g/L = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times g/L \text{ standard} \times F$$

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, F.

SAMPLE PREPARATION:

1. Sample dilution.

The amount of L-fucose present in the cuvette (i.e. in the 0.1 mL of sample being analysed) should range between 0.5 and 100 µg. The sample solution must therefore be diluted sufficiently to yield a concentration of L-fucose between 5 and 1000 mg/L.

Dilution table

Estimated concentration of L-fucose (mg/L)	Dilution with water	Dilution factor (F)
< 1000	No dilution required	1
1000-10000	1 + 9	10
>10000	1 + 99	100

If the value of $\Delta A_{L\text{-fucose}}$ is too low (e.g. < 0.100), weigh out more sample or dilute less strongly. Alternatively, the sample volume to be pipetted into the cuvette can be increased to 2.0 mL, making sure that the sum of the sample and distilled water components in the reaction is 2.10 mL and using the new sample volume in the equation.

2. Sample clarification.

a. Solutions:

Carrez I solution. Dissolve 3.60 g of potassium hexacyanoferrate (II) $\{K_4[Fe(CN)_6].3H_2O\}$ (Sigma cat. no. P9387) in 100 mL of distilled water. Store at room temperature.

Carrez II solution. Dissolve 7.20 g of zinc sulphate ($ZnSO_4.7H_2O$) (Sigma cat. no. Z4750) in 100 mL of distilled water. Store at room temperature.

Sodium hydroxide (NaOH, 100 mM). Dissolve 4 g of NaOH in 1 L of distilled water. Store at room temperature.

b. Procedure:

Pipette the liquid sample into a 100 mL volumetric flask which contains approx. 60 mL of distilled water, or weigh sufficient quantity of the sample into a 100 mL volumetric flask and add 60 mL of distilled water. Carefully add 5 mL of Carrez I solution, 5 mL of Carrez II solution and 10 mL of NaOH solution (100 mM). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

3. General considerations.

(a) Liquid samples: clear, slightly coloured and approximately neutral, liquid samples can be used directly in the assay.

(b) Acidic samples: if > 0.1 mL of an acidic sample is to be used undiluted, the pH of the solution should be increased to approx. 8.0 using 2 M NaOH, and the solution incubated at room temperature for 30 min.

(c) Carbon dioxide: samples containing carbon dioxide should be degassed by increasing the pH to approx. 8.0 with 2 M NaOH and gentle stirring, or by stirring with a glass rod.

(d) Strongly coloured samples: if used undiluted, strongly coloured samples should be treated by the addition of 0.2 g of polyvinylpolypyrrolidone (PVPP)/10 mL of sample. Shake the tube vigorously for 5 min and then filter through Whatman® No. 1 filter paper or centrifuge at 4,000 x *g* for 10 min.

(e) Solid samples: homogenise or crush solid samples in distilled water and filter if necessary.

(f) Samples containing fat: extract such samples with hot water at a temperature above the melting point of the fat, e.g. in a 100 mL volumetric flask at 60°C. Adjust to room temperature and fill the volumetric flask to the mark with distilled water. Store on ice or in a refrigerator for 15-30 min and then filter. Discard the first few mL of filtrate and use the clear supernatant (which may be slightly opalescent) for assay.

(g) Samples containing protein: deproteinise samples containing protein by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Filter or centrifuge at 1,500 *g* for 10 min and adjust the pH of the supernatant to approx. 8.0 with 1 M KOH. Use the supernatant in the assay after appropriate dilution.

SAMPLE PREPARATION EXAMPLES:

(a) Determination of L-fucose in plant samples.

Mill plant materials to pass a 0.5 mm screen. Weigh out 1.0 g of sample and extract with 90 mL of water (heated to 80°C). Quantitatively transfer to a volumetric flask and dilute to the mark with distilled water. Mix, filter and use the appropriately diluted, clear solution for the assay.

(b) Determination of L-fucose in fermentation samples and cell culture medium.

Incubate an aliquot (approx. 10 mL) of the solution at approx. 90-95°C for 10 min to inactivate enzyme activity. Centrifuge or filter and use the supernatant or clear filtrate (diluted according to the dilution table, if necessary) for the assay. Alternatively, deproteinisation can be performed with Carrez reagents. Homogenise gelatinous agar media with water and treat further as described above.

(c) Determination of L-fucose in polysaccharides and fibrous plant material.

Mill plant material or polysaccharide to pass a 0.5 mm screen using a Retsch centrifugal mill, or similar. Accurately weigh approx. 100 mg of material into a Corning® screw-cap culture tube (16 x 125 mm). Add 5 mL of 1.3 M HCl to each tube and cap the tubes. Incubate the tubes at 100°C for 1 h. Stir the tubes intermittently during the incubation. Cool the tubes to room temperature, carefully loosen the caps and add 5 mL of 1.3 M NaOH. Quantitatively transfer the contents of the tube to a 100 mL volumetric flask using distilled water and adjust the volume to 100 mL with distilled water. Mix thoroughly by inversion and filter an aliquot of the solution through Whatman® No. 1 filter paper or centrifuge at 1,500 *g* for 10 min.

(d) Determination of L-fucose in whole blood samples.

a. Solutions:

Concentrated Carrez I solution. Dissolve 30 g of potassium hexacyanoferrate (II) $\{K_4[Fe(CN)_6].3H_2O\}$ (Sigma cat. no. P9387) in 200 mL of distilled water. Store at room temperature.

Concentrated Carrez II solution. Dissolve 60 g of zinc sulphate $\{ZnSO_4.7H_2O\}$ (Sigma cat. no. Z4750) in 200 mL of distilled water. Store at room temperature.

b. Procedure:

Heat 1 mL of whole blood sample at approx. 80°C for 20 min in a microfuge tube then centrifuge at 13,000 x *g* for 10 min and recover the supernatant. Add 20 µL Carrez Reagent II and mix thoroughly, then add 20 µL Carrez Reagent I and mix thoroughly. Centrifuge the sample again at 13,000 x *g* for 10 min and recover the clarified supernatant for use in the assay. If required, dilute the sample appropriately in distilled water for the assay.

NOTE: The final volume of the clarified supernatant will be approx. one quarter of the starting volume of the original sample. Therefore adjust the volume of the starting material as required to obtain sufficient volume of clarified sample for the test.

(e) Determination of L-fucose in biological tissue samples.

Accurately weigh approx. 5 g of representative biological tissue into a 100 mL Duran® bottle. Add 20 mL of 1 M perchloric acid and homogenise for 2 min using an Ultra-turrax® or Polytron® homogeniser (or equivalent). Quantitatively transfer to a 40 mL glass beaker and adjust the pH to approx. 8.0 using 2 M KOH. Quantitatively transfer to a 100 mL volumetric flask and adjust to the mark with distilled water (ensuring the fat containing layer is "above" the mark, and the aqueous layer is "at" the mark). Store on ice for

20 min to precipitate potassium perchlorate and allow separation of the fat (if present). Centrifuge an appropriate volume of the sample at 13,000 x *g* for 10 min and recover the clarified supernatant for use in the assay, alternatively filter through Whatman® No. 1 filter paper, discarding the first 3-5 mL, and use the clear filtrate for the assay. If required, dilute the sample appropriately in distilled water for the assay.

NOTE: The amount of starting material and volumes used can be adjusted accordingly depending on the amount of analyte present in the sample.

(f) Determination of L-fucose in biological fluid samples (e.g. urine and serum).

For some biological fluid samples it may be sufficient to test them directly without any sample preparation other than appropriate dilution in distilled water. If this is not adequate then deproteinisation with either perchloric acid or trichloracetic acid may be required.

Deproteinise biological samples by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Centrifuge an appropriate volume of the sample at 1,500 x *g* for 10 min and recover the supernatant for use in the assay, alternatively filter through Whatman® No. 1 filter paper, discarding the first 3-5 mL, and use the filtrate for the assay. If required, dilute the sample appropriately in distilled water for the assay. Alternatively, use 50% (w/v) trichloroacetic acid instead of perchloric acid.

REFERENCE:

Morris, J. B. (1988). L-Fucose. "*Methods of Enzymatic Analysis*" (Bergmeyer, H. U., ed.), 3rd ed., **Vol.VI**, pp. 386-398, VCH Publishers (UK) Ltd., Cambridge, UK.

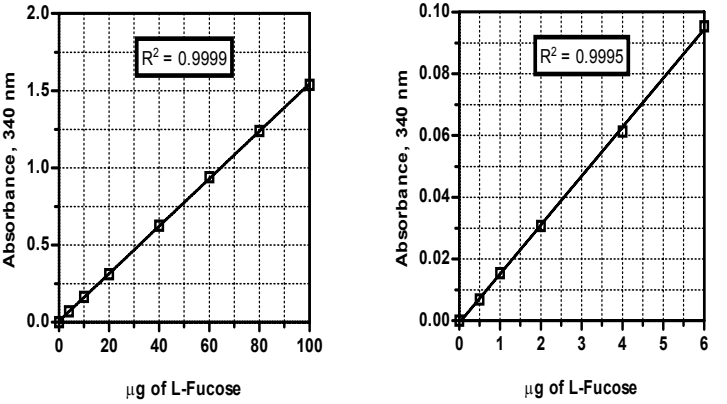

Figure 2. Calibration curves showing the linearity of **K-FUCOSE** from 0-100 µg of L-fucose. The reactions used to generate this calibration curve were performed at 37°C using 1 cm path-length cuvettes (Manual Format; page 4).

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