Maltose/Sucrose/ D-Glucose

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

UV-method

for the determination of maltose, sucrose and D-glucose in foodstuffs and other materials

Cat. No. 11 113 950 035

Test-Combination for 15 determinations each

For *in vitro* use only

Store at 2-8°C

For recommendations for methods and standardized procedures see references (2)

Principle (Ref. 1)

Maltose and sucrose are hydrolyzed in the presence of the enzyme α-glucosidase (maltase) at pH 6.6 to two molecules D-glucose or to D-glucose and D-fructose, respectively (1,2).

(1) Maltose + H_2O $\xrightarrow{\alpha\text{-glucosidase}}$ 2 D-glucose

(2) Sucrose + H_2O $\xrightarrow{\alpha\text{-glucosidase}}$ D-glucose + D-fructose

Moreover, sucrose is also hydrolyzed by the enzyme β-fructosidase (invertase) at pH 4.6 to D-glucose and D-fructose (3).

(3) Sucrose + H_2O $\xrightarrow{\beta\text{-fructosidase}}$ D-glucose + D-fructose

At pH 7.6 the enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) under simultaneous formation of adenosine-5'-diphosphate (ADP) (4).

(4) D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

The formed D-glucose-6-phosphate (G-6-P) is oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (5).

(5) G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phophate + NADPH + H^+

The amount of NADPH formed in this reaction is stoichiometric to the amount of sucrose, D-glucose and half the amount of maltose. The increase in NADPH is measured by means of its light absorbance at 334, 340 or 365 nm.

The Test-Combination contains

1. Bottle 1 with approx. 0.2 g lyophilizate, consisting of: citrate buffer, pH approx. 6.6; α-glucosidase, approx. 210 U
2. Bottle 2 with approx. 0.5 g lyophilizate, consisting of: citrate buffer, pH approx. 4.6; β-fructosidase, approx. 720 U
3. Bottle 3 with approx. 7.2 g powder mixture, consisting of: triethanolamine buffer, pH approx. 7.6; NADP, approx. 110 mg; ATP, approx. 260 mg; magnesium sulfate
4. Bottle 4 with approx. 1.1 ml suspension, consisting of: hexokinase, approx. 320 U; glucose-6-phosphate dehydrogenase, approx. 160 U

Preparation of solutions

1. Dissolve contents of bottle 1 with 6 ml redist. water.
2. Dissolve contents of bottle 2 with 10 ml redist. water.
3. Dissolve contents of bottle 3 with 45 ml redist. water.
4. Use contents of bottle 4 undiluted.

Stability of reagents

The contents of bottles 1, 2 and 3 are stable for at 2-8°C (see pack label).
Solution 1 is stable for 4 weeks at 2-8°C, for 2 months at −15 to −25°C. Bring solution 1 to 20-25°C before use.
Solution 2 is stable for 4 weeks at 2-8°C, for 2 months at −15 to −25°C. Bring solution 2 to 20-25°C before use.
Solution 3 is stable for 4 weeks at 2-8°C, for 2 months at −15 to −25°C. Bring solution 3 to 20-25°C before use.
The contents of bottle 4 are stable at 2-8°C (see pack label).

Procedure

Wavelength[1]: 340 nm, Hg 365 nm or Hg 334 nm
Glass cuvette[2]: 1.00 cm light path
Temperature: 20-25°C
Final volume: 3.020 ml
Read against air (without a cuvette in the light path) or against water
Sample solution: 4-100 µg maltose + sucrose + D-glucose/assay[3] (in 0.100-0.700 ml sample volume)

Pipette into cuvettes	Blank maltose sample	Maltose sample	Blank sucrose sample	Sucrose sample	Blank D-glucose sample	D-Glucose sample
solution 1*	0.200 ml	0.200 ml	-	-	-	-
solution 2*	-	-	0.200 ml	0.200 ml	-	-
sample solution**	-	0.100 ml	-	0.100 ml	-	0.100 ml
Mix*, and incubate for 20 min at 20-25°C. Add:						
solution 3	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
redist. water	1.800 ml	1.700 ml	1.800 ml	1.700 ml	2.000 ml	1.900 ml
Mix***, read absorbances of the solutions after approx. 3 min (A_1). Start reaction by addition of:						
suspension 4	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix***, wait for the completion of the reaction (10-15 min) and read absorbances of the solutions (A_2). If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbance increases constantly.						

* Pipette solution 1, solution 2 and sample solution, each, onto the bottom of the cuvette and mix by gentle swirling. When using a plastic spatula, remove it from the cuvette only directly before measuring absorbance A_1.

** Rinse the enzyme pipette or the pipette tip of the piston pipette with sample solution before dispensing the sample solution.

*** For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

If the absorbances increase constantly, extrapolate the absorbances A_2 to the time of addition of suspension 4 (HK/G6P-DH).

Determine absorbance differences (A_2-A_1) for blanks and samples. Subtract the absorbance differences of the blanks from the absorbance differences of the corresponding samples.

$$\Delta A = (A_2\text{-}A_1)_{sample} - (A_2\text{-}A_1)_{blank}$$

It follows:

$\Delta A_{maltose\ sample}$, $\Delta A_{sucrose\ sample}$ and $\Delta A_{D\text{-}glucose\ sample}$.

The difference of $\Delta A_{maltose\ sample}$ and $\Delta A_{sucrose\ sample}$ gives $\Delta A_{maltose}$.

The difference of $\Delta A_{sucrose\ sample}$ and $\Delta A_{D\text{-}glucose\ sample}$ gives $\Delta A_{sucrose}$.

The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently precise results (see "Instructions for performance of assay" and "Sensitivity and detection limit", pt.4).

1 The absorption maximum of NADPH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm **or** 334 nm.
2 If desired, disposable cuvettes may be used instead of glass cuvettes.
3 See instructions for performance of assay

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Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000\ (\times 2)^*} \times \Delta A\ [g/l]$$

V = final volume [ml]

v = sample volume [ml]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path [cm]

ε = extinction coefficient of NADPH at:
340 nm = 6.3 [l × mmol^{-1} × cm^{-1}]
Hg 365 nm = 3.5 [l × mmol^{-1} × cm^{-1}]
Hg 334 nm = 6.18 [l × mmol^{-1} × cm^{-1}]

It follows for maltose:

$$c = \frac{3.020 \times 342.3}{\varepsilon \times 1.00 \times 0.100 \times 1000 \times 2} \times \Delta A_{maltose} = \frac{5.169}{\varepsilon} \times \Delta A_{maltose}$$ [g maltose/l sample solution]

for sucrose:

$$c = \frac{3.020 \times 342.3}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{sucrose} = \frac{10.34}{\varepsilon} \times \Delta A_{sucrose}$$ [g sucrose/l sample solution]

for D-glucose:

$$c = \frac{3.020 \times 180.16}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D\text{-}glucose} = \frac{5.441}{\varepsilon} \times \Delta A_{D\text{-}glucose}$$ [g D-glucose/l sample solution]

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

When analyzing solid and semi-solid samples which are weighed out for sample preparation, the result is to be calculated from the amount weighed:

$$Content_{maltose} = \frac{c_{maltose}\ [g/l\ sample\ solution]}{weight_{sample}\ in\ g/l\ sample\ solution} \times 100\ [g/100\ g]$$

$$Content_{sucrose} = \frac{c_{sucrose}\ [g/l\ sample\ solution]}{weight_{sample}\ in\ g/l\ sample\ solution} \times 100\ [g/100\ g]$$

$$Content_{D\text{-}glucose} = \frac{c_{D\text{-}glucose}\ [g/l\ sample\ solution]}{weight_{sample}\ in\ g/l\ sample\ solution} \times 100\ [g/100\ g]$$

1. Instructions for performance of assay

The amount of maltose + sucrose + D-glucose present in the assay has to be between 8 µg and 100 µg (measurement at 365 nm) or 4 µg and 50 µg (measurement at 340, 334 nm), respectively. In order to get a sufficient absorbance difference, the concentration of maltose (inclusive sucrose and D-glucose) in the sample solution used for the assay should range between 0.10-1.0 g/l (measurement at 365 nm) or 0.05-0.5 g/l (measurement at 340, 334 nm). Dilute, if necessary.

Dilution table

Estimated amount of maltose + sucrose + D-glucose per liter measurement at		Dilution with water	Dilution factor F
340 or 334 nm	365 nm		
< 0.5 g	< 1.0 g	-	1
0.5-5.0 g	1.0-10 g	1 + 9	10
5.0-50 g	10-100 g	1 + 99	100
> 50 g	> 100 g	1 + 999	1000

If the measured absorbance difference (ΔA) is too low (e.g. < 0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) *or* the sample volume to be pipetted into the cuvette can be increased up to 0.700 ml (maltose assay), 1.800 ml (sucrose assay), 2.000 ml (D-glucose assay), resp.. The volume of water added must then be reduced so as to obtain the same final volume in the assays for sample and blank. The new sample volume v must be taken into account in the calculation

2. Technical information

If the ratio maltose + sucrose to D-glucose in the sample is higher than e.g. 10:1, the precision of the determination of maltose and sucrose is impaired. In this case, as much as possible of the D-glucose should be removed by means of glucose oxidase in the presence of oxygen from the air. (For details see pt. 10)

3. Specificity (Ref. 1)

α-Glucosidase hydrolyzes α-glucosidic bonds in maltose, maltotriose, sucrose, turanose, 2-O-α-D-glucosido-D-erythrose and maltitol (4-O-α-D-glucopyranosyl-D-sorbitol). Other α-glucosides such as α,α-trehalose, maltopentaose and higher oligo-glucosides, dextrins and starch do not react. Under the given reaction conditions, maltotetraose reacts to approx. 5%, isomaltose to 15% and palatinose (O-α-D-glucopyranosyl(1→6)-D-fructofuranose) to 40%. Carbohydrates with β-glucosidic bonds (lactose, lactulose, cellobiose), as well as raffinose are not hydrolized.

β-Fructosidase hydrolyzes the β-fructosidic bond in sucrose and other oligoglucosides. If the sample contains only sucrose, it is measured specifically via D-glucose. Even in the presence of fructosanes, sucrose can be measured specifically if after enzymatic hydrolysis with β-fructosidase D-glucose and D-fructose are determined and the ratio of these monosaccharides is 1:1. If the D-fructose rate dominates 2 β-oligofructosanes are contained in the sample . "Polyfructose" (e. g. inulin) is not split.

The determination of D-glucose is specific.

In the analysis of commercial sucrose, results of 100% have to be expected. In the analysis of water-free D-glucose (molecular weight 180.16) resp. D-glucose monohydrate (molecular weight 198.17) and of maltose, results of < 100% have to be expected because the materials absorb moisture. (Commercial maltose may also contain D-glucose.)

4. Sensitivity and detection limit (Ref. 1.2)

The smallest differentiating absorbance for the procedure in the determination of D-glucose is 0.005 absorbance units. This corresponds to a maximum sample volume v = 2.000 ml and measurement at 340 nm of a D-glucose concentration of 0.2 mg/l sample solution (if v = 0.100 ml, this corresponds to 4 mg/l sample solution).

The detection limit of 0.4 mg D-glucose/l is derived from the absorbance difference of 0.010 (as measured at 340 nm) and a maximum sample volume v = 2.000 ml.

The smallest differentiating absorbance for the procedure in the determination of maltose is 0.010 absorbance units (in the presence of D-glucose). This corresponds in the determination of maltose to a maximum sample volume v = 0.700 ml and measurement at 340 nm of a maltose concentration of 1 mg/l sample solution (if v = 0.100 ml, this corresponds to 8 mg/l sample solution).

The detection limit of 2 mg maltose/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume v = 0.700 ml.

The smallest differentiating absorbance for the procedure in the determination of sucrose is 0.010 absorbance units (in the presence of D-glucose). This corresponds to a maximum sample volume v = 1.800 ml and measurement at 340 nm of a sucrose concentration of 1 mg/l sample solution (if v = 0.100 ml, this corresponds to 15 mg/l sample solution).

The detection limit of 2 mg sucrose/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume v = 1.800 ml.

5. Linearity

Linearity of the determination exists from 4 µg maltose + sucrose + D-glucose/assay (2 mg maltose + sucrose + D-glucose/l sample solution; sample volume v = 1.800 ml) to 100 µg maltose + sucrose + D-glucose/assay (1 g maltose + sucrose + D-glucose/l sample solution; sample volume v = 0.100 ml).

6. Precision

In a double determination of D-glucose using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to a D-glucose concentration of approx. 4-8 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.04-0.08 g/100 g can be expected.)

* Only when analyzing maltose

In a double determination of maltose, resp. sucrose using one sample solution, a difference of 0.010 to 0.015 absorbance units may occur in the presence of D-glucose in the sample. With a sample volume of v = 0.100 ml and measurement at 340 nm, this corresponds to approx. 8–12 mg maltose/l or 15–25 mg/sucrose/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor F. If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 10 g/l, a difference of 0.08- 0.12 g maltose/100 g or 0.15–0.25 g sucrose/100 g can be expected.)

The following data for the determination of maltose have been published in the literature:

CV = 1.7–2.1 % maltose solutions (Ref. 1.2)

Rusks for children:

x = 2.9 g/100 g r = 0.260 g/100 g $s_{(r)}$ = ± 0.092 g/100 g
R = 0.461 g/100 g $s_{(R)}$ = ± 0.163 g/100 g (Ref. 2.3)

7. Recognizing interference during the assay procedure

7.1 If the conversion of D-glucose has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.

7.2 On completion of the reaction, the determination can be restarted by adding D-glucose (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.

The reaction cannot be restarted with maltose and sucrose as, subsequent to altering the reaction conditions from pH 6.6 to pH 7.6, resp. from pH 4.6 to 7.6 ("change of the buffer"), maltose and sucrose are no longer cleaved.

7.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.

When analyzing solid samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.

The use of "single" and "double" sample volumes in double determinations is the simplest method of carrying out a control assay in the determination of maltose and sucrose.

7.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample **and** assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.

7.5 Possible losses during the determination can be recognized by carrying out recovery tests: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.

8. Reagent hazard

The reagents used in the determination of maltose, sucrose and D-glucose are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.

After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.

9. General information on sample preparation

In carrying out the assay:
Use **clear, colorless and practically neutral liquid samples** directly, or after dilution according to the dilution table, and of a volume up to 2.000 ml (D-glucose), resp. up to 0.700 ml (maltose), resp. up to 1.800 ml (sucrose);
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Adjust **acid samples** to pH 8 by adding sodium or potassium hydroxide solution (determination of D-glucose);
Adjust **acid and weakly colored samples** to approx. pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min (determination of D-glucose);
Measure **"colored" samples** (if necessary adjusted to approx. pH 8) against a sample blank (= buffer or redist. water + sample), adjust the photometer to 0.000 with the blank in the beam (determination of D-glucose);
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with polyamide, e.g. 1 g/100 ml;
Crush or homogenize **solid or semi-solid samples**, extract with water or dissolve in water and filter if necessary; resp. remove turbidities or dyestuffs by Carrez clarification;
Deproteinize **samples containing protein** with Carrez reagents;
Extract **samples containing fat** with hot water (extraction temperature should be above the melting point of the fat involved). Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15 min and filter; alternatively clarify with Carrez-solutions after the extraction with hot water.

Carrez clarification:
Pipette the liquid sample into a 100 ml volumetric flask which contains approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g $K_4[Fe(CN)_6] \times 3\ H_2O$/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g $ZnSO_4 \times 7\ H_2O$/100 ml). Adjust to pH 7.5–8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.

Samples containing protein should not be deproteinized with perchloric acid or with trichloroacetic acid in the presence of sucrose and maltose as these disaccharides are fully or partially hydrolized with the release of D-glucose. The Carrez clarification is recommended for normal use.

10. Special preparation of sample for the determination of maltose and sucrose in the presence of a large excess of D-glucose

The precision of the maltose and sucrose determination is impaired if the ratio of D-glucose to maltose and sucrose is higher than e.g. 10:1. In this case, as much as possible of the D-glucose should be removed. In the presence of glucose oxidase (GOD) and oxygen from the air, D-glucose is oxidized to D-gluconate:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{D-gluconate} + H_2O_2$$

Hydrogen peroxide is destroyed by catalase:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

Reagents

Glucose oxidase (GOD), from Aspergillus niger, 200 U/mg (25°C; D-glucose as substrate); amylase and β-fructosidase < 0.01% each
Catalase
Triethanolamine hydrochloride,
$MgSO_4 \times 7\ H_2O$
NaOH, 4 M

Preparation of solutions for 10 determinations

Enzyme solution:
Dissolve 5 mg (approx. 1000 U) GOD in 0.750 ml redist. water, add 325 KU catalase (from bovine liver, 25°C, H_2O_2 as substrate) and mix.

Buffer solution:
Dissolve 5.6 g triethanolamine hydrochloride and 0.1 g $MgSO_4 \times 7\ H_2O$ in 80 ml redist. water, adjust to pH 7.6 with NaOH (4 M), and fill up to 100 ml with redist. water.

Stability of solutions

The enzyme solution must be prepared freshly daily.
The buffer solution is stable for 4 weeks when stored at 2–8°C.

Performance of D-glucose oxidation

Pipette into 10 ml volumetric flask	
buffer solution sample solution (up to approx. 0.5% D-glucose) enzyme solution	2.000 ml 5.000 ml 0.100 ml
Pass a current of air (O_2) through the mixture for 1 h; during the oxidation process **check the pH with indicator paper and, if necessary, neutralize the formed acid with NaOH**.	

To inactivate the enzymes GOD and catalase, keep the volumetric flask in a

boiling water-bath for 15 min, allow to cool, and dilute to the mark with water. Mix and filter, if necessary. Use the clear solution for the determination of maltose and sucrose. Determine the residual D-glucose in a parallel assay and subtract as usual.

11. Further applications

The method may also be used in research when analyzing biological samples. For sample treatment see Ref. 1.2.

References

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2.1 Gombocz, E., Hellwig, E., Vojir, F. & Petuely, F. (1981) Deutsche Lebensmittel-Rundschau **77**, 10-11 (Maltose), 11 (Saccharose) und 3 (D-Glucose)

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2.3 Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG; Untersuchung von Lebensmitteln: Bestimmung von Maltose in Kinder-Zwieback und Zwiebackmehl, 48.02.07-2 (Mai 1985)

3.1 Drawert, F. & Hagen, W. (1970) Enzymatische Analysenmethoden zur Bestimmung von Würze- und Bierinhaltsstoffen: II. Bestimmung von Maltose neben Saccharose, Glucose und Fructose in Würze und Bier; Verhalten der Zucker während der Gärung und Lagerung, Brauwissenschaft **23**, 95-101

3.2 Postel, W., Drawert, F. & Hagen, W. (1977) Enzymatische Differenzierung der Zucker in Malz-, Nähr- und Süßbieren: I. Enzymatische Analyse von Glucose, Fructose, Saccharose und Maltose, Deutsche Lebensmittel-Rundschau **67**, 107-110 und 195-202 (II. Zusammensetzung und Beurteilung)

For further references, see Test-Combination Sucrose/D-Glucose (Cat. No. 10 139 041 035).

For further information see instructions for

Test-Combination D-Glucose	**Cat. No. 10 716 251 035**
Test-Combination D-Glucose/D-Fructose	**Cat. No. 10 139 106 035**
Test-Combination Sucrose/D-Glucose	**Cat. No. 10 139 041 035**
Test-Combination Sucrose/D-Glucose/D-Fructose	**Cat. No. 10 716 260 035**
Test-Combination D-Sorbitol/Xylitol	**Cat. No. 10 670 057 035**
Test-Combination Starch	**Cat. No. 10 207 748 035**

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