Glycerol

BOEHRINGER MANNHEIM / R-BIOPHARM
Enzymatic BioAnalysis / Food Analysis

UV-method

for the determination of glycerol in foodstuffs and other materials.

For use in foodstuff hygiene only. **Store at 2-8°C**

Cat. No. 10 148 270 035

Test-Combination for 3 × 11 determinations

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

Principle (Ref. 1)

Glycerol is phosphorylated by adenosine-5'-triphosphate (ATP) to L-glycerol-3-phosphate in the reaction catalyzed by glycerokinase (GK) (1).

(1) Glycerol + ATP $\xrightarrow{GK}$ L-glycerol-3-phosphate + ADP

The adenosine-5'-diphosphate (ADP) formed in the above reaction is reconverted into ATP by phosphoenolpyruvate (PEP) with the aid of pyruvate kinase (PK) with the formation of pyruvate (2).

(2) ADP + PEP $\xrightarrow{PK}$ ATP + pyruvate

In the presence of the enzyme L-lactate dehydrogenase (L-LDH), pyruvate is reduced to L-lactate by reduced nicotinamide-adenine dinucleotide (NADH) with the oxidation of NADH to NAD (3).

(3) Pyruvate + NADH + H^+ $\xrightarrow{L\text{-}LDH}$ L-lactate + NAD^+

The amount of NADH oxidized in the above reaction is stoichiometric to the amount of glycerol. NADH is determined by means of its light absorption at 334, 340 or 365 nm.

The Test-Combination contains

1. Three bottles 1, with approx. 2 g coenzyme/buffer mixture each, consisting of:
   glycylglycine buffer, pH approx. 7.4; NADH, approx. 7 mg; ATP, approx. 22 mg; PEP-CHA, approx. 11 mg; magnesium sulfate
2. Bottle 2 with approx. 0.4 ml suspension, consisting of:
   pyruvate kinase, approx. 240 U; L-lactate dehydrogenase, approx. 220 U
3. Bottle 3 with approx. 0.4 ml glycerokinase suspension, approx. 34 U
4. Bottle 4 with glycerol assay control solution for assay control purposes (measurement of the assay control solution is not necessary for calculating the results.) Use the assay control solution undiluted. (Expiry date: see pack label)

Preparation of solutions for 10 determinations

1. Dissolve contents of one bottle 1 with 11 ml redist. water. Before use allow the solution to stand for approx. 10 min at 20-25°C.
2. Use contents of bottle 2 undiluted.
3. Use contents of bottle 3 undiluted.

Stability of reagents

The contents of the bottles 1 are stable at 2-8°C (see pack label).
Solution 1 is stable for 4 days at 2-8°C.
Bring solution 1 to 20-25°C before use.
The contents of bottles 2 and 3 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: 1-40 µg glycerol/assay[3] (in 0.100-2.000 ml sample volume).

Pipette into cuvettes	Blank	Sample
solution 1	1.000 ml	1.000 ml
sample solution*	-	0.100 ml
redist. water	2.000 ml	1.900 ml
suspension 2	0.010 ml	0.010 ml
Mix**, **wait for completion of the pre-reaction[4]** (approx. 5-7 min) and read the absorbances of the solutions (A_1). Start reaction by addition of:		
suspension 3	0.010 ml	0.010 ml
Mix**, wait for completion of the reaction (approx. 5-10 min) and read the absorbances of blank and sample immediately one after another (A_2). If the reaction has not stopped after 15 min, continue to read the absorbances at 2 min intervals until the absorbance decreases constantly over 2 min.		

* 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 absorbance A_2 decreases constantly, extrapolate the absorbances to the time of the addition of suspension 3 (GK).

Determine the absorbance differences (A_1-A_2) for both, blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample.

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

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).

If the absorbance difference of the sample (ΔA_{sample}) is higher than 1.000 (measured at 340 nm, Hg 334 nm resp.) or 0.500 (measured at Hg 365 nm) respectively, the concentration of glycerol in the sample solution is too high. The sample is to be diluted according to the dilution table in that case.

Calculation

According to the general equation for calculating the concentration:

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

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 NADH at:
340 nm = 6.3 [l × $mmol^{-1}$ × cm^{-1}]
Hg 365 nm = 3.4 [l × $mmol^{-1}$ × cm^{-1}]
Hg 334 nm = 6.18[l × $mmol^{-1}$ × cm^{-1}]

It follows for glycerol

$$c = \frac{3.020 \times 92.1}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A = \frac{2.781}{\varepsilon} \times \Delta A \text{ [g glycerol/l sample solution]}$$

If the sample has been diluted during 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:

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

1 The absorption maximum of NADH 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
4 **It is necessary to wait for completion of this pre-reaction (ADP in ATP and pyruvate in PEP react), otherwise the results will be too high.**

0817.**10249041001**®

1. Instructions for performance of assay

The glycerol content present in the assay has to be between 1 µg and 40 µg. In order to get a sufficient absorbance difference, the sample solution is diluted to yield a glycerol concentration between 0.04 and 0.4 g/l.

Dilution table

Estimated amount of glycerol per liter	Dilution with water	Dilution factor F
< 0.4 g	-	1
0.4-4.0 g	1 + 9	10
4.0-40 g	1 + 99	100
> 40 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 2.000 ml. 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

It is necessary to wait for the completion of the pre-reaction after the addition of suspension 2 (PK/L-LDH).

3. Specificity (Ref. 1)

The method is specific for glycerol. Dihydroxyacetone is not converted under the given conditions.

In the analysis of commercial glycerol results of approx. 100% (calculated on the water-free substance) have to be expected.

4. Sensitivity and detection limit

The smallest differentiating absorbance for the procedure is 0.005 absorbance units. This corresponds to a maximum sample volume v = 2.000 ml and measurement at 340 of a glycerol concentration of 0.1 mg/l sample solution (if v = 0.100 ml, this corresponds to 2 mg/l sample solution).

The detection limit of 0.4 mg/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume v = 2.000 ml.

5. Linearity

Linearity of the determination exists from approx. 1 µg glycerol/assay (0.4 mg glycerol/l sample solution; sample volume v = 2.000 ml) to 40 µg glycerol/assay (0.4 g glycerol/l sample solution; sample volume v = 0.100 ml).

6. Precision

In a double determination 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 glycerol concentration of approx. 2-5 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 0.02-0.05 g/100 g can be expected.)

The following data have been published in the literature:

CV = 2.5 %	in series	serum	
CV = 2.59 %	from day to day	serum	(Ref. 1.2)
Pastilles	m = 1.482 g/100 g	r = 0.0393 g/100/g	R = 0.1484 g/100 g
Standard	m = 0.206 g/l	r = 0.0047 g/l	R = 0.0115 g/l
White grape juice	m = 0.613 g/l	r = 0.0138 g/l	R = 0.0337 g/l
Red grape juice	m = 0.907 g/l	r = 0.0251 g/l	R = 0.0511 g/l
White wine	m = 6.050 g/l	r = 0.1154 g/l	R = 0.5002 g/l
Red wine	m = 16.57 g/l	r = 0.3166 g/l	R = 1.1240 g/l
			(Ref. 3.8)

7. Interference/sources of error

The slow hydrolysis of ATP and phosphoenolpyruvate as well as the air oxidation of NADH results in a slow creep reaction which can be taken into account by extrapolation. An extrapolation is not absolutely necessary if the absorbances of blank and sample are measured immediately one after another.

8. Recognizing interference during the assay procedure

8.1 If the conversion of glycerol has been completed according to the time given under "Procedure" it can be concluded in general that no interference has occurred.

8.2 On completion of the reaction, the determination can be restarted by adding glycerol (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.

8.3 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.

8.4 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.

8.5 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.

9. Reagent hazard

The reagents used in the determination of glycerol contain hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulations 67/548 and 99/45 and subsequent alteration, supplementation and adaptation guidelines. Please refer to the safety date sheet or the labels of the affected vials for further information.

10. 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;
Filter **turbid solutions**;
Degas **samples containing carbon dioxide** (e.g. by filtration);
Adjust **acid samples** to approx. pH 8 by adding sodium or potassium hydroxide solution;
Adjust **acid and weakly colored samples** to approx. pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min;
Treat **"strongly colored" samples** that are used undiluted or with a higher sample volume with polyvinylpolypyrrolidone (PVPP) or with polyamide, e.g. 1g/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.

11. Application examples

Determination of glycerol in fruit juices
Dilute the sample to yield a glycerol concentration of less than 0.4 g/l (see dilution table).

Filter turbid juices. Use the clear solution for the assay, even if it is slightly colored.

When analyzing **strongly** colored juices (e.g., sour cherry juice, red grape juice), decolorize the sample as follows:

Mix 10 ml of juice and approx. 0.1 g of polyamide powder or polyvinylpolypyrrolidone (PVPP), stir for 1 min and filter. Use the clear solution, which may be slightly colored, for the assay.

Determination of glycerol in wine
Dilute the sample according to the dilution table.

In general, red wine can also be analyzed without decolorization.

Determination of glycerol in beer
To remove the carbonic acid, stir about 5-10 ml of beer for approx. 1 min using a glass rod or filter; dilute the largely CO_2-free sample according to the dilution table.

Determination of glycerol in marzipan
Remove chocolate coating of the marzipan if necessary. Accurately weigh approx. 1 g of marzipan into a small porcelain cup containing approx. 2 g sea-sand, grind thoroughly, mix with approx. 50 ml water and incubate at approx. 60°C for 20 min. Pour supernatant solution into a 100 ml volumetric flask. Wash the residue (sea-sand) twice with portions of 10 ml water each and transfer the wash solution into the volumetric flask. Allow the solution in the volumetric flask to cool to 20-25°C and fill up to the mark with water. For separation of fat, place in a refrigerator for 15 min. Filter the solution, centrifuge, if necessary, at 3000 rpm. Use the largely clear solution for the assay, dilute, if necessary (see dilution table).

Determination of glycerol in tobacco products
Mix and mince sample thoroughly (grain size max. 0.2 mm). Accurately weigh approx. 1 g into a 100 ml volumetric flask. After addition of approx. 70 ml water stir vigorously (magnetic stirrer) for approx. 1 h at 20-25°C. Fill up to the mark with water, mix and filter.

Pipette 25 ml filtrate into a 50 ml volumetric flask, add successively and mix after each addition: 5 ml Carrez-I-solution (3.60 g $K_4[Fe(CN_6)] \times 3\ H_2O$/100 ml), 5 ml Carrez-II-solution (7.20 g $ZnSO_4 \times 7H_2O$/100 ml) and 10 ml NaOH (0.1 M). Fill up to the mark with water, mix and filter. Use the filtrate for the assay (0.100-0.500 ml).

12. Further applications

The method may also be used in the examination of paper (Ref. 2.1), cosmetics (Ref. 4.1), pharmaceuticals (Ref. 4.5, 4.6), and in research when analyzing biological samples. For details of sampling, treatment and stability of the sample see Ref. 1.2.

12.1 Determination of glycerol in cosmetics

Determination of glycerol in skin tonic
Dilute sample as far as the glycerol concentration lies under 0.4 g/l. Use undiluted or diluted sample for the assay.

Determination of glycerol in pre-shave, after-shave
If the after-shave is mixable with water without turbidity, proceed according to skin tonic.

If a turbidity occurs after diluting the after-shave with water, this turbidity has to be removed with polyamide or activated charcoal:

Mix 1.0 ml after-shave with 9.0 ml water, add 100 mg polyamide or activated charcoal, mix again and filter (dilution factor: 10).

If the glycerol concentration in the filtrate is lower than 0.02 g/l, the sample volume which has to be pipetted into the assay can be increased up to 2.000 ml. The quantity of water which has to be added must be reduced accordingly.

Determination of glycerol in skin cream
Accurately weigh approx. 1 g skin cream into a 100 ml volumetric flask, add approx. 70 ml water and keep at 60°C for 30 min, while occasionally shaking. After cooling to 20-25°C, fill up to the mark with water. Place volumetric flask in a refrigerator or better in an ice-bath for 15 min. Filter or centrifuge solution. If necessary dilute filtrate or supernatant and use for the assay.

Determination of glycerol in toothpaste
Accurately weigh approx. 1 g toothpaste into 100 ml beaker, add approx. 70 ml water and extract for 30 min at 60°C while stirring (heatable magnetic stirrer). Transfer suspension into centrifuge tube. Pour the clear supernatant into a 250 ml volumetric flask after centrifugation. Rinse precipitate with water into a beaker and repeat extraction one to two times. Fill up the volumetric flask to the mark, filter, if necessary.

Depending on the glycerol concentration use clear solution, respectively the filtrate directly or after dilution with water for the determination. If the glycerol concentration in the clear solution or in the filtrate is lower than 0.02 g/l the sample volume, which has to be pipetted into the assay, can be increased up to 2.000 ml. The water quantity, to be added, has to be reduced accordingly.

Alternatively the Carrez clarification may be used for the preparation of the toothpaste sample.

Determination of glycerol in soap
Accurately weigh approx. 1 g grated soap into a beaker, add approx. 50 ml HCl (0.1 M) and while stirring rigorously incubate on a heatable magnetic stirrer until boiling. Transfer aqueous phase with a pipette into a 100 ml volumetric flask. Repeat extraction with approx. 30 ml HCl (0.1 M).

Bring volumetric flask to 20-25°C and fill up to the mark with redist. water. Place volumetric flask in an ice-bath or refrigerator for 15 min. Filter through a fluted filter. Use filtrate, depending on the expected glycerol concentration, diluted or undiluted for the determination.

If the glycerol concentration is lower than 0.02 g/l, the volume which has to be pipetted into the assay can be increased up to 2.000 ml. In this case the volume of the water quantity to be added has to be reduced accordingly.

12.2 Determination of glycerol in fermentation samples and cell culture media

Place the sample (after centrifugation, if necessary) in a water-bath at 80°C for 15 min to stop enzymatic reactions. Centrifuge and use the supernatant (diluted according to the dilution table, if necessary) for the assay. Alternatively, deproteinization can be carried out with perchloric acid or with Carrez-solutions. See the above-mentioned examples.

Homogenize gelantinous agar media with water and treat further as described.

References

1.1 Kreutz, F.H. (1962) Enzymatische Glycerinbestimmung, Klin. Wochenschrift **40,** 362-363

1.2 Eggstein, M. & Kuhlmann, E. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Aufl., Bd. 2, S. 1871-1877, Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 4, pp. 1825-1831; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

1.3 Wieland, O.H. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H. U. ed.) 3rd ed., vol. VI, pp. 504-510, Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel

2.1 Untersuchung von Papieren, Kartons und Pappe für Lebensmittel-Verpackungen (gem. Empf. XXXVI der Kunststoffkommission des Bundesgesundheitsamtes) Kapitel 8 (Methoden), Pkt. 3.5.2. (März 1979)

2.2 Methodenbuch für Weinanalysen in Österreich (1980), herausgegeben von Arbeitsgemeinschaft der Landw. Versuchsanstalten in Österreich (ALVA)

2.3 Gombocz, E., Hellwig, E., Vojir, F. & Petuely, F. (1981) Deutsche Lebensmittel-Rundschau **77,** 8

2.4 Brautechnische Analysenmethoden, Band III, S. 553-556 (1982), Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK), herausgegeben von F. Drawert im Selbstverlag der MEBAK, Freising

2.5 Schweizerisches Lebensmittelbuch, Kapitel 61B (Enzymatische Bestimmungen)/2.2 (1985), Kapitel 28A (Frucht- und Gemüsesäfte, Fruchtnektare, Fruchtsirupe, Konzentrate und Pulver)/12.3 (1994), Kapitel 30 (Wein)/40 (1967), Kapitel 30A (Wein)/40 (1973)

2.6 Recueil des méthodes internationales d'analyse des vins et des moûts, Complément n° 1 à l'édition officielle de juin 1990, OFFICE INTERNATIONAL DELA VIGNE ETDUVIN, S. 149-151

3.1 Drawert, F. & Kupfer, G. (1963) Enzymatische Analysen, I. Mitteilung: Bestimmung von Glycerin in Weinen und Traubenmosten, Zeitschrift für Lebensmittel-Untersuchung und -Forschung, **123,** 211-217

3.2 Berner, G. & Guhr, G. (1969) Freies Glycerin und Gesamt-Glycerin in Monoglyceriden und in Partialester-Gemischen; ein Vergleich der enzymatischen Glycerin-Bestimmung mit chemischen Methoden, Fette-Seifen-Anstrichmittel-Die Ernährungsindustrie **71,** 459-464

3.3 Holbach, B. & Woller, R. (1976) über den Zusammenhang zwischen Botrytisbefall von Trauben und den Glycerin- sowie Gluconsäuregehalt von Wein, Weinwissenschaft **31,** 202-214

3.4 Holbach, B. & Woller, R. (1977) Vergleichende Glycerinbestimmung im Wein nach der Methode Rebelein und der enzymatischen Methode, Weinwissenschaft **32,** 212-218

3.5 Wagner, K. & Kreutzer, P. (1978) Beitrag zur Glycerinbestimmung in Wein, Likörwein und weinhaltigen Getränken, Weinwissenschaft **33,** 109-113

3.6 Mändl, B., Wullinger, F., Fischer, A. & Piendl, A. (1970) Weitere Ergebnisse über den Gehalt verschiedener Biersorten an Glycerin, Pyruvat, Citrat und Malat, Brauwissenschaft **23,** 11-18

3.7 Klopper, W.J., Angelino, S.A.G.F., Tuning, B. & Vermeire, H.A. (1986) Organic acids and glycerol in beer, J. Inst. Brew. **92,** 225-228

3.8 Walter, E. & Kohler, P. (1985) Ringversuch für die enzymatische Bestimmung von Glycerin, Z. Lebensm. Unters. Forsch. **180,** 121-125

3.9 Plessi, M., Monzani, A., & Coppini, D. (1988) Determination of the Monosaccharide and Alcohol Content of Balsamic and Other Vinegars by Enzymatic Methods, Agric. Biol. Chem. **52,** 25-30

3.10 Tamminga-Groenveld, T., Westra, A.M. & Strikwerda, K. (1989) Enzymatisch en Hogedruk Vloeistofchromatografisch Onderzoek van de Gehalten aan Glycerol in Jenever, Vieux en Beerenburg, De Ware(n)-Chemicus **19,** 109-120

3.11 Huidobro, J.F., Rea, M.E., Branquinho de Andrade, P.C., Sancho, M.T., Muniategui, S. & Simal-Lozano, J. (1993) Enzymatic determination of glycerol in honey, J.Agric.Food Chem. **41,** 557-559.

3.12 Russmann, H. (1998) Hefen und Glycerin in Blütenhonigen - Nachweis einer Gärung oder einer abgestoppten Gärung, Lebensmittelchemie, **52,** 116-117

4.1 Henniger, G. & Boos, H. (1978) Anwendung der enzymatischen Analyse bei der Untersuchung kosmetischer Präparate, dargestellt an einigen Beispielen; Seifen - Öle - Fette - Wachse **104,** 159-164

4.2 Schmidt, F. H. & von Dahl, K. (1968) Zur Methode der enzymatischen Neutralfettbestimmung in biologischem Material, Z. Klin. Chem. Klin. Biochem. **96,** 156-159

4.3 Wieland, O. (1957) Biochem. Z. **329,** 313

4.4 Wieland, O. & Witt, I. (1974), Dihydroxyacetone, in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.) 3. Auflage, Bd. 2, S. 1487, Verlag Chemie, Weinheim, and Wieland, O. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) 2nd ed., vol. 4, pp. 1404; Verlag Chemie, Weinheim/Academic Press, Inc., New York and London

4.5 Michal, G. (1976) Enzymatische Analyse in der Pharmazie, Acta Pharmaceutica Technologica, Suppl. 1, S. 151-162

4.6 Pfandl, A. & Menschig, D. (1984) Ein Beitrag zur enzymatischen Glycerin- und Ethanolbestimmung, Pharm. Ind. **46,** 403-407

Glycerol assay control solution (Bottle 4)

Concentration: see bottle label

Glycerol assay control solution is a stabilized aqueous solution of glycerol. It is used as an assay control solution for the enzymatic determination of glycerol in foodstuffs and other sample materials.

Application:

1. Addition of glycerol assay control solution to the assay mixture:
The assay control solution is used for the determination instead of the sample solution.

2. Restart of the reaction, quantitatively:
After completion of the reaction with sample solution and measuring of A_2, add 0.050 ml assay control solution to the assay mixture. Read absorbance A_3 after the end of the reaction (approx. 15 min). Calculate the concentration from the difference of (A_2-A_3) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by addition of the assay control solution, the result differs insignificantly from the data stated on the bottle label.

3. Internal standard:

The assay control solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances:

Pipette into cuvettes	Blank	Sample	Standard	Sample + Standard
solution 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml
suspension 2	0.010 ml	0.010 ml	0.010 ml	0.010 ml
sample solution	-	0.100 ml	-	0.050 ml
assay control sln.	-	-	0.100 ml	0.050 ml
redist. water	2.000 ml	1.900 ml	1.900 ml	1.900 ml
Mix, and read absorbances of the solutions (A_1) **after approx. 7 min.** Continue as described in the pipetting scheme under “Procedure”. Follow the instructions given under “Instructions for performance of assay” and the footnotes.				

The recovery of the standard is calculated according to the following formula:

$$\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times 100\ [\%]$$

R-BIOPHARM AG
An der neuen Bergstraße 17
D-64297 Darmstadt
Phone + 49 61 51 / 81 02-0
Fax + 49 61 51 / 81 02-20
www.r-biopharm.com