ENZYTEC™ *fluid* Ammonia — Id-N°: 5390

Test kit for 4 x 10 determinations (For in vitro use only)

Reagent for photometric determination of Ammonia in food stuff and other sample material

Method

Enzymatic UV-method with glutamate dehydrogenase (GLDH).

Principle

2-Oxoglutarate + NH_4^+ + NADH $\xrightarrow{GLDH}$ L-Glutamate + NAD^+ + H_2O

Thanks to the clarification reagents integrated in the test kit, milk samples can be tested after 1:10 dilution (see under sample preparation).

Storage instructions and reagent stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and if contamination is avoided. Do not freeze the reagents!

Warnings and precautions

1. The reagents contain sodium azide (0.95 g/l) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

Reagent preparation

The reagents are ready-to-use.

Materials required but not provided

Dist. water (aseptic, free of heavy metal) and usual laboratory equipment.

Package content and concentration of the reagents

R1	**4 x 20,0 ml**	Buffer	pH 7,8
		ADP	0,75 mmol/l
		GLDH	≥ 30 KU/l
		Clearing reagents	
R2	**4 x 5,0 ml**	NADH	≥ 1,3 mmol/l
R3	**4 x 5,0 ml**	Buffer	pH 8,0
		2-Oxoglutarate	60 mmol/l

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure.

- Use clear, colourless and practically neutral liquid samples directly, or after dilution to an Ammonia concentration between 10 – 70 mg/l.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples. Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water. Filtrate or clarify if necessary.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter.
- Adjust acid samples by adding KOH /NaOH, or alkaline samples with HCl, until approx. pH 8 is reached.
- Treat strongly coloured samples with Polyvinylpolypyrrolidone (PVPP e.g. 1 g/100 ml Sample), or measure each sample with sample blank (SB), instead of using reagent blank (see assay procedure).
- **Milk** can be used after pre-dilution with the normal assay procedure (1+9 dilution, e.g 500 µl milk + 4500 µl water). This dilution factor must be included in the calculation.
- **The Carrez clarification cannot be used because Ammonia is unstable under the alkaline conditions of the Carrez reaction.**

Assay procedure

Wavelength: 340 nm, Hg 334 nm, Hg 365 nm
Optical path: 1 cm
Temperature: 20 – 25 °C / 37 °C
Measurement: against air or against water

	Reagent blank (RB)	Sample	Sample blank (SB, optional)
Sample / standard	-	100 µl	100 µl
Dist. water	100 µl	-	-
Reagent 1	2000 µl	2000 µl	2000 µl
Reagent 2	500 µl	500 µl	500 µl
Mix, incubate for 5 min. at 37 °C or 15 min. at 20 - 25 °C, read absorbance A1, then add:			
Reagent 3	500 µl	500 µl	-
Dist. Water	-	-	500 µl
Mix, wait until the end of the reaction (incubation for approx. 5 min. at 37 °C or approx. 15 min. at 20 - 25 °C) and read absorbance A2.			

For the manual procedure above, reagent blank must be performed for every run, and subtracted during calculation of results. Sample blank is performed only when interferences by the sample itself are suspected.

Application sheets for automated systems are available on request.

Note:
R1 and R2 can be used pre-mixed as one single reagent. Mix 4 parts of R1 with 1 part of R2 (e.g. 20 ml R1 + 5 ml R2) and use 2500 µl of the mixture for the determination. The stability of this mixture is 1 week at 2 – 8°C. The reagent mixture must be protected from light!

Calculation

Measurement with RB only: $\Delta A = (df \times A_1 - A_2)_{sample} - (df \times A_1 - A_2)_{RB}$

or with SB: $\Delta A = (df \times A_1 - A_2)_{sample} - (df \times A_1 - A_2)_{SB} - (df \times A_1 - A_2)_{RB}$

With df = dilution factor of optical densities, because of reagent volumes:
df = (sample volume + R1+ R2) / (sample vol. + R1 + R2 + R3) = 0.839.

Calculation formula:

$$C_{ammonia}\,[g/l\ sample\ sol.] = \frac{V \times MW \times \Delta A}{\varepsilon \times d \times v \times 1000}$$

with:

V	(Total volume)	= 3100 [µl]
MW	(Molecular weight Ammonia)	= 17.03 [g/mol]
d	(Optical path)	= 1.00 [cm]
v	(Sample volume)	= 100 [µl]
ε	(Extinction coefficient NADH)	[l x $mmol^{-1}$ x cm^{-1}] :
	340 nm = 6.3	334 nm = 6.18 365 nm = 3.4

Here from results for the determination at:

340 nm:	$C_{ammonia}$ [g/l]	= 0,0838 x ΔA
334 nm		= 0,0854 x ΔA
365 nm		= 0,1553 x ΔA

The above factors have to be recalculated again when changing parameters, e.g. the sample volume.

Dilution factors of the sample preparation have to be considered in the calculation.

Calculation in solid samples:

$$Content_{Ammonia}\,[g/100\ g] = \frac{C_{Ammonia}\,[g/l\,]}{weight_{sample}\,[g/l\ sample\ solution]} \times 100$$

Calibration / assay control

For the calibration of automated photometric systems, and for internal quality control of precision and accuracy, it is necessary to prepare a fresh ammonia solution. Weigh precisely 25 mg Ammonium sulfate $[(NH_4)_2SO_4$; MW = 132.1 g/mol] into a 100 ml volumetric flask, and fill-up to the mark. The solution has an ammonia concentration of 64 mg/l (if weight of ammonium sulfate = 24 mg, then ammonia = 24 x 64/25 = 61.4 mg/l). The standard must be used fresh.

Performance characteristics

Measuring range

The test has been developed to determine Ammonia concentrations between 10 and 70 mg/l (measured at 340 nm). When values exceed this range, samples should be diluted into this range with dist. water. The dilution factor has to be considered in the calculation.

Specificity

The test is specific for Ammonia. Interferences are not known.

Lowest detection limit

0.3 mg/l, measured at 340 nm.
The lowest detection limit is the smallest Ammonia concentration differentiating from zero. It is calculated out of three standard deviations from 20 replicates of a zero sample.

Waste Management

Please refer to local legal requirements.

Manufacturer

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