PURPOSE OF THE TEST

Yeasts need nitrogen to grow. One of the main sources of nitrogen are the proteins, peptides and amino acids present in the medium (primary amines, PAN); the other main source is the ammonium ion itself. The determination prior to the fermentation of the amount of assimilable nitrogen makes it possible to adjust it adequately to avoid unexpected stops of the fermentation process due to nitrogen deficit.

METHOD

Glutamate dehydrogenase (GLDH) catalyzes the condensation of ammonia and α-ketoglutarate in L-Glutamate in the presence of NADH.

$$NH_4^+ + \alpha\text{-}Ketoglutarate + NADPH \xrightarrow{GLDH} L\text{-}Glutamate + NADP^+$$

The variation in absorbance due to the disappearance of NADPH measured at 340 nm is proportional to the concentration of ammonia.

CONTENT

R1	2 x 30 mL	HEPES buffer 100 mM, pH 8.0, NADPH 0.5 mM, preservatives
R2	1 x 15 mL	GLDH (>100 U/mL), α-Ketoglutarate 20 mM
CTRL	1 x 3 mL	Ammonia control 125 mg NH_4^+/L (110-140 mg/L)

REAGENT PREPARATION

Reagents are ready to use and are stable up to expiry date as supplied when stored at 2-8 ºC. Do not freeze.

Discard if absorbance of blank is lower than 1.000 OD at 340 nm.

SAMPLES

The samples must be free of turbidity and particles. Centrifuge or filter if necessary. The presence of CO_2 introduces instability in the measure. Samples containing CO_2 must be degassed beforehand. In samples with very high colour intensity, the pigment may interfere with the measurement. Treat with polyvinylpolypyrrolidone (PVPP 0.1 g for each 10 mL) to reduce the level of colour. Samples with concentration higher than the measurement range must be diluted accordingly with distilled water. Multiply the final result by the dilution factor.

PROCEDURE OVERVIEW

Treat standard, controls and samples as Sample. Use distilled water as Blank.

Use WINECONTROL (code SD2200) or WINECALRTU (code SY2100R) as standard.

Volumes stated below can be adjusted to other analytical procedures. Expected performance can vary if those ratios S:R1:R2 are not used exactly as stated.

Pipette into a cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 1	720 µL	720 µL
Distilled water	9 µL	--
Sample/Standard	--	9 µL

Mix, incubate at 37ºC for 1 minutes and read absorbance at 340 nm (A_1). Then add into the cuvette:

	Blank reaction	Sample/Std Reaction
Reagent 2	180 µL	180 µL

Mix, incubate for 1 minutes at 37ºC and read absorbance at 340 nm (A_2).

Concentration of ammonia is calculated as:

$$Ammonia = \frac{(A_2 - 0.80xA_1)_{\text{sample}} - (A_2 - 0.80xA_1)_{\text{blank}}}{(A_2 - 0.80xA_1)_{\text{standard}} - (A_2 - 0.80xA_1)_{\text{blank}}} \; x\; C \text{ g/L}$$

Factor 0.80 is used to correct absorbance for dilution after adding reagent 2. C is the value of concentration stated in the standard label for ammonia.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	AMMONIA	
Method	End Point A	
Direction	Decreasing	
Main Wavelength	340	
Sec. Wavelength	--	
Sample	3	
Reagent 1	240	
Reagent 2	60	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	3 - 4
Reading cycle [150 | 240]	20 - 21	31 - 32
Units	mg/L	
Decimals	0.0	
Measure range	14 ~ 260	
R1 Lim. Abs	10000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

* Concentration in WINECALRTU standard (SY2100R) is expressed in mgN/L and mg NH_4/L

Procedure is linear up to 260 mg NH_4^+/L. Calibrate with a single point using the highest concentration standard or with several points as per your quality procedures.

PERFORMANCE

Limit of quantification (LoQ): 14 mg N/L
Limit of linearity: 260 mg N/L

NOTES

It is recommended to use the control included in the kit to verify the quality of the calibration. Each laboratory must establish its own acceptance criteria, as well as the necessary corrective actions in case of rejection.

Yeast available nitrogen (YAN) is calculated as:

$$YAN = [NH_4^+] \text{ mg N/L} + [\text{PAN}] \text{ mg N/L}$$

Units for ammoniacal nitrogen could also be expressed as:

$$[NH_4^+] \text{ mg N/L} = [NH_4^+] \text{ mg } NH_3 \text{ /L x 0,82}$$

$$[NH_4^+] \text{ mg N/L} = [NH_4^+] \text{ mg } NH_4^+ \text{ /L x 0,78}$$

REFERENCES

1. Compendium of International methods of analysis – OIV, Vol 1&2 (2008).
2. Bermeyer, HU. Methods of Enzymatic Analysis, 2nd Ed. Vol. 1, p. 112-117. Academic Press, Inc. NY.

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