SD6008: PRIMARY AMINO NITROGEN (PAN)

COLORIMETRIC – OPA/NAC

PURPOSE OF THE TEST

Yeasts need nitrogen to grow. Some 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

The groups of the primary amines react specifically with o-Phthaldialdehyde (OPA) and N-acetylcysteine (NAC) to form a chromophore that present an absorption peak at 340 nm. The increase in absorbance at 340 nm is proportional to the concentration of primary amines. Cyclic amines (proline and hydroxyproline) and free ammonium are not detected by this method.

CONTENT

R1	2 x 24 mL	Buffer pH 9.4, preservatives
R2	2 x 8 mL	N-Acetylcysteine, preservatives
R3	1 x 16 mL 	Buffer pH 9.4, o-Phthaldialdehyde, preservatives *WARNING: H226: Flammable liquid and vapour. H315: Causes skin irritation. H319: Causes serious eye irritation. P262: Do not get in eyes, on skin, or on clothing. P302+P352: IF ON SKIN: Wash with plenty of water. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.*
STD	1 x 3 mL	Aminoacids (Glu) 125 mg N/L (106 – 144 mg N/L)

REAGENT PREPARATION

Reagent 1: pour the content of one vial of R1B into a bottle of R1A. Mix gently avoiding foam formation. Wait at least 20 minutes before use. This mix is stable up to 6 months at 2-8 ºC and avoiding contamination. Do not freeze.

Reagent 2: Use R2. Reagent is ready to use and stable until expire date. Store at 2-8 ºC and avoid contamination. Do not freeze

Discard if absorbance of blank is higher than 0.300 OD at 340 nm.

Standard is ready to use.

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 0.1g 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	600 µL	600 µL
Distilled water	15 µL	--
Sample/Standard	--	15 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	150 µL	150 µL

Mix, incubate for 10 minutes at 37ºC and read absorbance at 340 nm (A_2).

Concentration of primary amine groups is calculated as:

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

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 PAN.

ASSAY PARAMETERS FOR ANALYZER DIONYSOS®

Dionysos model	**150**	**240**
Name	PAN	
Method	End Point A	
Direction	Increasing	
Main Wavelenght	340	
Sec. Wavelenght	--	
Sample	5	
Reagent 1	200	
Reagent 2	50	
Calibration	Linear	
Blank cycle [150 | 240]	3 - 4	4 - 5
Reading cycle [150 | 240]	21 - 22	31 - 32
Units	mg/L	
Decimals	0	
Measure range	4 ~ 250	
R1 Lim. Abs	3000	
Ratio Dil. Auto.	--	
Vol. Sample Dil. Auto	--	

* Concentration in WINECALRTU standard (SD2200) is expressed in mg N/L

Procedure is linear up to 250 mg N/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): 4 mg N/L
Limit of linearity: 250 mg N/L

NOTES

It is recommended to use wine controls to verify quality of calibration. Each laboratory should establish its own quality criteria for acceptance, as well as proper corrective action procedures in case of rejection.

Yeast available nitrogen (YAN) is calculated as:

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

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. (1974).
3. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Wine analysis and production. Van Nostrand Reinhold, 1st Ed. (1990).