YAN Test Kit
User Manual

The Vinmetrica YAN (Yeast Assimilable Nitrogen) Test Kit (Part number 200-14) provides a simple and accurate way to determine YAN levels in wine, must and other samples.

Materials provided in the kit:

1. YAN Reagent (37% Formaldehyde/15% methanol, Part Number SC-200-14-1)
   NOTE: see cautions on page 2!
2. YAN Formaldehyde Neutralizer Powder (Sulfite, Part Number SC-200-14-2)
3. Three Plastic Transfer Pipettes (SC-100-5)
4. 15 mL conical tube (SC-100-5)

Things you will need:

1. Vinmetrica SC-200 or SC-300 with pH electrode, or comparable pH meter (0.02 pH resolution required).
2. Vinmetrica pH 4.01 and pH 7.00 Reference Solutions, or equivalent solutions for pH calibration.
3. Vinmetrica TA Titrant and accessories provided in the SC-200 or SC-300 Kit.
4. Distilled water (DI water), which can be found at most grocery stores.
5. It's handy to have a wash bottle for rinsing, like Vinmetrica Part Number SC-100-17.

Why Test for YAN?

Yeast assimilable nitrogen is an important nutrient in fermentation of wine. If levels are too low, fermentation may stop prematurely, and/or off odors can develop (mostly from generation of hydrogen sulfide – "rotten egg" smell). In this case you may want to add supplements like DAP or other yeast nutrients before and/or during fermentation. Many yeast and nutrient manufacturers follow a study by the UC Davis Department of Viticulture and Enology relating optimal nitrogen levels (in milligrams of nitrogen per liter, mg N/L) to brix level at harvest:

21°Bx = 200 mg N/L	25°Bx = 300 mg N/L
23°Bx = 250 mg N/L	27°Bx = 350 mg N/L

How it works:

A wine sample is titrated to pH 8.2 with TA Titrant (this gives the TA value as a bonus). Then the YAN reagent (formaldehyde) is added. This causes any amino groups or ammonia to become formylated, releasing one proton per amine/ammonia and lowering the pH. The sample is then titrated back to 8.2 as before. The YAN value is calculated from the volume of TA Titrant used in the second titration.

CAUTION! The YAN reagent (formaldehyde and methanol components) is flammable. Keep away from sparks and open flame. The YAN reagent is TOXIC and a possible CARCINOGEN!

Do not inhale fumes or ingest any solutions containing it. Do not pipet by mouth! Perform all steps with formaldehyde in a fume hood or similar well-vented environment. Wear laboratory gloves and safety glasses at all times when handling formaldehyde. If contact with skin or clothes occurs, flush with plenty of water and apply a solution of the formaldehyde neutralizer powder (1 tsp/50 mL water).

Procedure:

1. Clarify wine or must sample if needed by decanting, filtration or centrifugation. De-gas any excess dissolved CO_2 by taking about 15 mL of sample into a suitable container and shaking until effervescence ceases.
2. Calibrate your pH electrode in the usual manner.
3. Check and adjust pH of the YAN Reagent:

   Pour off enough YAN Reagent into the 15 mL conical tube for your immediate needs. You need 1 mL per sample. Take a minimum of 3 mL and maximum of 5 mL at a time.

   Rinse your pH electrode, remove excess water, then place in the 15 mL conical with formaldehyde and swirl. If the pH is below 7, use a plastic transfer pipette or eyedropper to add 1 drop TA Titrant, mix well and check again. Repeat this process carefully until the pH gets up to between 7.5 and 8.5 (2-4 drops typically is needed - If you're having trouble hitting the target, you can dilute the TA Titrant: add 1 big drop (about 0.05 mL) to about 0.5 mL water, mix, and use this dropwise once you get above pH 6.). If the pH goes above 8.7 you will need to bring it back down. In this case you can add a little more of the formaldehyde solution, or dropwise 0.1 M HCL solution (can make this by adding 1 mL of your SO_2 Acid Solution to 20 mL water)
4. **Optional:** If you suspect your wine sample has high free SO_2 levels (>150 ppm), you may need to precipitate it with barium chloride[1]. Otherwise proceed to the next step.
5. Take a 10.0 mL degassed wine sample into a 100 mL beaker or similar vessel; add 10 mL water and do a TA titration (check your SC-200 or SC-300 manual), but note that since you are using a 10 mL sample rather than the usual 5 mL, it will probably require more than 5 mL of TA Titrant to reach the endpoint of pH 8.2 – 8.3. If the end point goes above pH 8.5, repeat the step. The TA value can now be calculated if desired[2], but is not needed to complete the test.
6. **Do not pipette by mouth!** Using another of your plastic transfer pipettes, add 1 mL of YAN Reagent that has been pH-adjusted as in step (3.). Mix well for about 1 minute.
7. The pH of the wine will drop back to around 6 to 7. Titrate back to 8.2 with the TA Titrant (be sure to note starting and ending volumes on your burette or syringe!).
8. Measure the volume in mL, 'V', of the TA Titrant used in step (7.).

$$\text{YAN, mg N/L} = V * 0.133 * 1400 \qquad \text{[Eq. 1.]}$$

Note: Typical values for YAN are 50 to 500 mg/L

[1] Add 1 mL 1 N $BaCl_2$ to a 10 mL wine sample, mix and let stand 15 minutes. Bring to 20 mL with water. Filter through Whatman no. #1 paper. Titrate as in step 5.

[2] In this case, g/L Tartaric acid = mL TA Titrant used, i.e., do not multiply by 2, because you took twice as much sample.

Finishing Up:

1. Clean-up: To neutralize any residue of formaldehyde, add ¼ teaspoon (about 1.5 g) of the YAN Formaldehyde Neutralizer Powder for every 1 mL formaldehyde used. Mix well and allow to stand for at least 1 hour. Dispose in accordance with local regulations with plenty of water.
2. Turn off your pH meter and rinse and store the electrode properly per its manual's instructions.
3. Reagents should be stored at room temperature out of direct sunlight and away from children.
4. Rinse all plastic ware with DI water.

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1. The materials provided in the kit, as described on page 1 above, ("Materials") are warranted as follows: All reagents and non-reagent accessories are warranted against defects in workmanship for 6 months from date of purchase. The reagents are warranted to perform as described herein up until any stated expiration date or 6 months after purchase, whichever is later, provided storage recommendations are followed. THE WARRANTIES IN THESE TERMS AND CONDITIONS ARE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTIES OF MERCHANTABILITY, NONINFRINGEMENT, OR FITNESS FOR A PARTICULAR PURPOSE, SAID WARRANTIES BEING EXPRESSLY DISCLAIMED.

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All Materials offered by Vinmetrica are intended for use by individuals who are familiar with laboratory procedures and their potential hazards. The Materials contain chemicals which may be harmful if misused. Due care should be exercised with all Materials to prevent direct human contact. Glassware can break and chemicals can splash during experiments; ***always use safety glasses***. We strongly recommend using nitrile or latex gloves and wearing long pants, long sleeves and closed-toed shoes. Keep out of reach of children.

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