

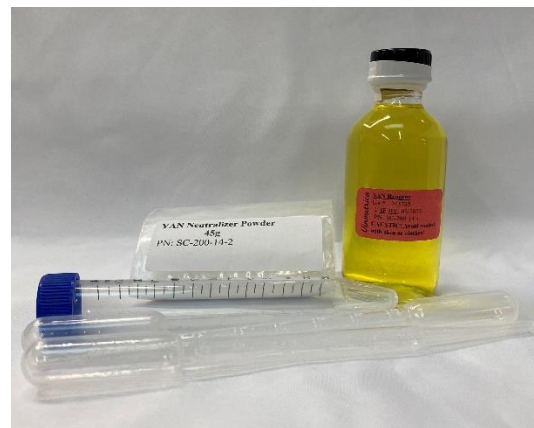


# YAN Test Kit User Manual

The Vinmetrica YAN (Yeast Assimilable Nitrogen) Test Kit 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, PN: SC-200-14-1)  
**NOTE: see cautions on page 2!**
2. YAN Formaldehyde Neutralizer Powder (Sulfite, PN: SC-200-14-2)
3. Three Plastic Transfer Pipettes (PN: SC-100-5)
4. 15 mL conical tube (PN: SC-50-7)



## 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 (i.e.: 5mL syringe) provided in the SC-200 or SC-300 Kit. (Note: we recommend using a 10 mL burette for best precision in dispensing the TA Titrant)
4. (Optional) Safety Pipetting Bulb for collecting wine sample. (PN: SC-300-16)
5. 25 or 10 mL burette
6. Distilled water (DI water), which can be found at most grocery stores.
7. Rinse Bottle PN: SC-100-17.

## Why Test for YAN?

Yeast assimilable nitrogen is an important nutrient in fermentation of wines, beers, and other juices. 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 wine’s 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 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 release one proton per amine/ammonia, 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). Note the neutralization instructions under “Finishing Up” below.**

## **Procedure:**

1. Clarify wine, must or other sample if needed by decanting, filtration or centrifugation. De-gas any excess dissolved CO<sub>2</sub> by taking about 15 mL of sample into a suitable container and shaking until outgassing ceases, or by applying a good vacuum with shaking for 30 sec.
2. Calibrate your pH electrode in the usual manner.
3. Adjust the pH of the YAN Reagent. As supplied, the YAN reagent is acidic and needs to be adjusted to approximately neutral pH. You can use your pH meter, or rely on the pH indicator included in the YAN reagent:
  - a. Pour off enough YAN Reagent into the 15 mL conical tube for your immediate needs. You need 2 mL per sample. Take a minimum of 5 mL and maximum of 10 mL at a time.
  - b. Fill your transfer pipette or, ideally, your 10 mL burette with the TA Titrant
  - c. Add TA titrant a single drop at a time, being sure to mix well between additions, until the YAN reagent suddenly turns from yellow-orange to magenta red. If you want to use your pH electrode, you are looking for the pH to be between 6.5 and 8.5.
4. **Optional:** If you suspect your sample has high free SO<sub>2</sub> levels (>150 ppm), you may need to precipitate it with barium chloride<sup>1</sup>. Otherwise proceed to the next step.
5. Take a 10.0 mL degassed wine sample and dispense 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<sup>2</sup>, but is not needed to complete the test. We recommend using a 10 mL burette for this titration; a 25 mL buret is also suitable.
6. **Do not pipette by mouth!** Using another of your plastic transfer pipettes, add 2 mL of YAN Reagent that has been pH-adjusted as in step (3.) to your wine sample in your beaker. Mix well for about 1 minute.
7. The pH of the wine will drop. Titrate back to 8.2 with the TA Titrant (be sure to note starting and ending volumes on your burette or syringe!).

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<sup>1</sup> Add 1 mL 1 N BaCl<sub>2</sub> to a 10 mL wine sample, mix and let stand 15 minutes. Bring to 20 mL with water. Filter through Whatman #1 paper or similar. Proceed with step 5, but adjust final calculation, as sample has been diluted by 10%.

<sup>2</sup> 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.

8. Measure the volume in mL, 'V', of the TA Titrant used in step (7.). Calculate YAN as:  
YAN, mg N/L (mg nitrogen per liter) = V \* 0.133 \* 1400 [Eq. 1.]  
(Note: Typical values for YAN are 50 to 500 mg/L in must.)

### **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.

## **WARRANTIES AND LIABILITIES**

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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### **HAZARDS AND TOXICITY**

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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