



Vinmetrica Residual Sugar Assay User Manual

The Vinmetrica Residual Sugar Assay Kit provides a simple, accurate and affordable way to determine Residual Sugar (glucose + fructose) concentrations in wine and other samples.

Materials provided in the kit:

1. Buffer Solution ***Keep in Refrigerator***
2. Reaction Buffer Solution or Powder ***Keep in Freezer***
3. 1N NaOH (Store at room temperature)
4. Standard Solution ***Keep in Freezer***
5. Diluent Solution ***Keep in Refrigerator***
6. Enzymatic Powder ***Keep in Freezer***



Figure 1. The Residual Sugar Reagent Kit

Things you will need:

1. Vinmetrica **Residual Sugar Assay Plastics Kit**. Available from Vinmetrica (Part Number: RS-2). It is highly recommended that the Plastics Kit be purchased with your **first** Residual Sugar Reagent Kit. The plastics only need to be purchased once and replaced if necessary. The Plastics Kit contains the following: Measuring scoop (for Enzymatic powder), one 1 mL serological pipette, one 10 mL serological pipette, one 5 mL serological pipette, one plastic transfer pipette, one 100 mL plastic beaker, and twelve plastic vials.
2. Vinmetrica SC-200 or SC-300 with pH electrode, or comparable pH meter (0.01 pH resolution required).
3. Vinmetrica pH 4.01 and pH 7.00 Reference Solutions, or equivalent solutions for pH calibration.
4. Distilled water (DI water), which usually can be found at your local grocery store. It's handy to have a wash bottle for rinsing. Rinse bottle available from Vinmetrica (Part number SC-100-17)
5. (Optional) Invertase Powder Vials (for measuring sucrose levels). Available from Vinmetrica (Part Number: RS-10)
6. (Optional) Additional plastic vials. Available from Vinmetrica (Part Number: RS-8)
7. (Optional) 25 mL Sampling Pipette (for reconstituting Reaction Buffer powder, Part number SC-100-4)

Why Test for Residual Sugar?

Residual sugar (RS) refers to any significant concentration of sugar that is contained in wine at the end of fermentation. Winemakers are typically most interested in knowing the concentration of the fermentable hexoses glucose and fructose (in some cases sucrose may be present as well). These determine the level of sweetness of the wine (see Appendix B for more information on sweetness characteristics). At residual sugar levels above around 2 g/L (0.2%), your wine can spontaneously restart fermentation unless it has been properly stabilized.

How it works:

The enzymes present in Vinmetrica's Enzymatic Powder convert residual sugars in wine into their 6-phosphate forms. This reaction gives off a hydrogen ion, thereby lowering the pH of the test solution. Therefore, the more residual sugar in the wine, the greater the drop in pH. By measuring the drop in pH of your wine sample and comparing that value to a known glucose standard, you can reliably calculate a residual sugar value for your wine. If you want to measure sucrose, you can add Invertase Powder (available separately, Part Number: RS-10) that will enable this measurement.

Assay Notes:

- The Reaction Buffer is usually provided as a freeze-dried powder that must be dissolved in 25 mL of water (see Setup section below). In some cases, the reaction buffer has been provided in liquid form ready to use.
- Vinmetrica's residual sugar assay has an incubation period of 20 minutes with approximately 10-20 minutes of sample preparation time.
- Most of the reagents of the kit must be stored in either a freezer (-5 to -20C) or refrigerator (+4C); please take note to store reagents properly.
- It is necessary to run one Residual Sugar Assay Standard and one Blank each time you use the kit, whether for one sample or multiple samples.
- The kit provides enough Enzymatic Powder for over 24 tests. This allows you to run up to 22 tests with at least one Residual Sugar Standard and one Blank. Note that you must always run a blank and a standard each time you assay one or more samples. With the 12 plastic vials provided, you may run up to 10 tests at one time, with 1 Standard and 1 Blank. If you would like to run all 22 tests at once, additional plastic reaction bottles will be needed. Additional bottles are available (Part Number: RS-8)
- Most wines made without added sucrose will not have measurable levels of this sugar in them. If you want to measure sucrose in a sample, obtain the Invertase Powder (Vinmetrica Part Number: RS-10), and follow one of the two possible sucrose methods in the Procedure (see steps 12, 15, and Appendix A.)
- Plastic vials may be cleaned with DI water after completion of assay and reused.

Setup

1. To prepare for this assay you will need to have your pH meter/probe calibrated and ready for measurement. We recommend that you follow your meter's procedure to calibrate at pH 7.00 and pH 4.01.
2. Take all reagents out of cold storage and allow them to come to room temperature.
3. **Prepare Reaction Buffer:** [NOTE: skip this step if your Reaction Buffer came in the form of a liquid.] Remove the crimp seal and rubber cap from the bottle of Reaction Buffer Powder. With a pipette or other accurate measuring device, dispense exactly 25 mL of DI water into the bottle (this amount fills the bottle nearly to level of the neck). Replace the rubber septum and swirl gently several times until all solids are dissolved to give a clear, orange tinted solution. Note: any unused portion of this solution may be stored up to 1 month in the freezer, but it is important to re-freeze it as soon as possible after use in steps 6 through 9 below.

Procedures

1. With the 5 mL serological pipette, measure exactly 3.0 mL of wine that is to be tested and place this in the 100 mL plastic beaker. Rinse the inside and outside of the 5 mL pipette with dH₂O into a waste container and drain thoroughly. Then use the pipette to measure 3.0 mL of Buffer solution into the 100 mL beaker with wine sample. This is your wine solution.
Note: You may want to remove the cotton plug in the end of pipette for ease of rinsing.
2. Put your pH probe (be sure it's calibrated!) in the wine solution and begin swirling or mixing. Read pH and, with plastic bulb pipette, carefully add 1 N NaOH into the wine solution one drop at a time. Continue to add 1 N NaOH until the pH of the wine solution is 8.20 ± 0.03 (i.e. 8.17 to 8.23). As you approach pH 7.5 it is important to only add one drop at a time as you may overshoot past 8.25. If you do go past 8.25 you may use the Acid Solution (small drops!) that is provided as part of the SO₂ kit to drop the pH back to 8.2; if you find it hard to hit pH 8.2 with the 1N NaOH, simply dilute the NaOH 5- to 10-fold with DI water and use this once you reach pH 7.5.
3. If your wine sample is a dry wine, proceed to step 5. If your wine is expected to be medium dry, medium, or sweet, you may need to further dilute the wine sample as in step 4. See Appendix B for guidance.
4. From the wine solution that was just pH'd to 8.2, use the 1 mL serological pipette to take exactly the amount indicated in Appendix B into a second 100 mL beaker or other container. With a 10 mL serological pipette add the exact amount of Diluent (Appendix B) to second container. Mix well. Reminder: This step is only required if assaying a medium dry, medium, or sweet wine.
5. With the wine solution at pH 8.2 (and further diluted if necessary) use the 1 mL serological pipette to add 0.5 mL of diluted wine sample to a clean plastic vial.
6. Rinse 1 mL serological pipette with dH₂O into a waste container, drain thoroughly, then measure exactly 1.0 mL of Reaction Buffer and add to the plastic vial.

7. Repeat steps 2-6 for all wine samples. We recommend that you label each individual wine sample.
8. To prepare the Standard use the clean 1 mL serological pipette and measure exactly 0.50 mL of Residual Sugar Standard; add this to a clean plastic vial. Rinse and drain the pipette thoroughly, then add exactly 1.0 mL of Reaction Buffer to this vial. Label this bottle with “Standard”.
9. For the blank, measure exactly 0.50 mL of Diluent and add this to a clean plastic vial. Rinse and drain the pipette thoroughly, then add exactly 1.0 mL of Reaction Buffer to this bottle. Label this bottle “Blank”.
10. You should now have at least three (3) plastic vials: a blank, a standard, and at least one sample. You may have more if you are running more wine samples. With all vials lined up and labeled, measure and record the initial pH. Note: Electrode will not be completely submerged in solution. By gently swirling solution with electrode contacting it, you should be able to obtain a stable pH value.
11. This value should be between 8.10 and 8.30. Record this measurement for each sample including standard and blank. Try to recover all the liquid off of the pH probe back into the sample bottle by gently flicking the probe a few times above the liquid level. Remove, then rinse the pH electrode thoroughly with DI water between each sample and remove excess water from the electrode tip by gently shaking or blotting it away.
12. With the initial pH recorded, you are now ready to begin your assay. Add 20 mg of Enzymatic Powder to each vial. This is the amount delivered by a level fill of the measuring scoop provided in the Plastics kit (see Figure 2). Immediately swirl the vial for 5 seconds to get good mixing. Repeat this step for each sample including Standard and Blank. [If you are measuring sucrose as a *combined value*, add one scoop of invertase powder now. See Appendix A]

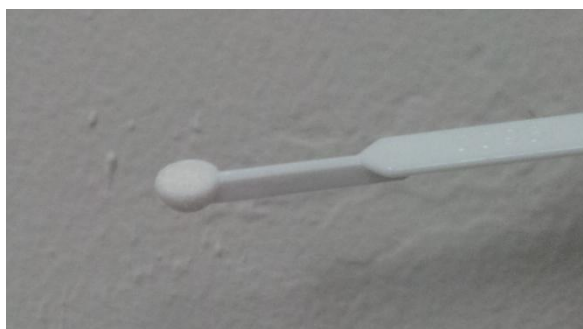


Figure 2. Measuring scoop with level fill (about 20 mg) of Enzymatic Powder .

13. Allow vials to sit at room temperature for 20 minutes; agitate gently at least once over this time.
14. At 20 minutes, measure pH of sample and record the value as in step 11. Repeat this step for all samples including the Standard and the Blank. The Standard and any wine samples containing residual sugar will see a drop in pH.
15. Skip this step if you are not measuring sucrose as a *separate value* (see Appendix A). If you are measuring sucrose as a *separate value*, add invertase powder now, mix well and allow to react 60 minutes. Meanwhile, proceed with the calculations in step 16-18 below for the non-sucrose

sugars. At the end of this second 60 minute period, take additional pH readings as in step 14. Refer to Appendix A to calculate the *separate value* for sucrose.

16. Now use the values you recorded and subtract them from each other. For example

$$\begin{aligned} \text{Initial pH} &= 8.27 & 60 \text{ min pH} &= 7.88 \\ \Delta &= 8.27 - 7.88 = 0.39 \end{aligned}$$

Repeat this subtraction step for all samples including standard and blank. Call these values Δ_{WS} (wine sample), Δ_{STD} (standard), and Δ_B (blank).

17. Plug these values into this equation:

$$\text{residual sugar, g/L} = 2 * (1.25 * ((\Delta_{WS} - \Delta_B) / (\Delta_{STD} - \Delta_B)))$$

This gives your residual sugar concentration in g/L. For example:

$$\text{if } \Delta_{WS} = 0.11 \quad \Delta_{STD} = 0.39 \quad \Delta_B = 0.07,$$

$$\text{then residual sugar} = 2 * (1.25 * ((0.11 - 0.07) / (0.39 - 0.07))) = 0.3 \text{ g/L}$$

18. If you further diluted your wine sample as in step 4, simply multiply your answer by the dilution factor. In the example above, if you had diluted the sample by a dilution factor of 100, your concentration of residual sugar would be 30 g/L. (Typically in sweet wines the residual sugar ranges between 50-150 g/L; this was used for example only).

Finishing Up:

1. Turn off your pH meter and rinse and store the electrode properly.
2. If you have unused solutions or Enzymatic Powder vials, store back immediately as directed on bottle. Especially be sure to place any unused Reaction Buffer in the freezer as soon as possible.
3. Rinse all plastic ware with DI water.

Technical assistance: info@vinmetrica.com tel. 760-494-0597

WARRANTIES AND LIABILITIES

1. The materials provided in the kit, as described on pages 1 and 2 above, (“Materials”) are warranted as follows: All reagents, powders 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.**
2. Buyer agrees that its sole and exclusive remedy against Vinmetrica shall be limited to the repair and replacement of Materials or parts of Materials, provided Vinmetrica is promptly notified in writing, prior to the expiration of the warranty period specified above, of any defect. Vinmetrica’s liability for any damages due Buyer shall be limited to the purchase price of the Materials.
3. **VINMETRICA’S MAXIMUM LIABILITY FOR ALL DIRECT DAMAGES, INCLUDING WITHOUT LIMITATION CONTRACT DAMAGES AND DAMAGES FOR INJURIES TO PERSONS OR PROPERTY, WHETHER ARISING FROM VINMETRICA’S BREACH OF THESE TERMS AND CONDITIONS, BREACH OF WARRANTY, NEGLIGENCE, STRICT LIABILITY, OR OTHER TORT WITH RESPECT TO THE MATERIALS, OR ANY SERVICES IN CONNECTION WITH THE MATERIALS, IS LIMITED TO AN AMOUNT NOT TO EXCEED THE PRICE OF THE MATERIALS. IN NO EVENT SHALL VINMETRICA BE LIABLE TO BUYER FOR ANY INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES, INCLUDING WITHOUT LIMITATION LOST REVENUES AND PROFITS.**

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.

Vinmetrica

1945 Camino Vida Roble, Suite I - Carlsbad, CA 92008

www.vinmetrica.com (760) 494-0597 info@vinmetrica.com

Technical assistance: info@vinmetrica.com tel. 760-494-0597

Copyright 2014-15. Sportsman Consulting, LLC DBA Vinmetrica. All rights reserved.

Appendix A: Measuring sucrose.

Although sucrose is not normally present in unsweetened grape musts and wines, it is sometimes added for various reasons. You can measure sucrose as a *combined value* with all fermentable sugars, or as a *separate value* from other sugars. Adding the Invertase Powder (available separately, Vinmetrica Part Number: RS-10) causes sucrose, which is not active in the assay, to be hydrolyzed into its fructose and glucose components, which are active.

Combined value: you will add invertase powder (10 mg, or one full rounded scoop, see Figure 2 above) along with the Enzyme powder at step 12 above. Proceed with the rest of the assay in the usual way (skip step 15 of course).

Calculation: follow steps 16-18 as usual; this produces a value that represents sucrose, glucose and fructose combined.

Separate value: you will add invertase powder (10 mg, or one full rounded scoop, see Figure 2 above) along with the Enzyme powder at step 15 above.

Calculation: Proceed with the calculation of residual sugar as in steps 16-18, using the initial pH values for the original sample and the final pH value after the second (Invertase) 60 minute period. This will produce a value for combined sugars.

Subtract the value for residual (non-sucrose) sugar, calculated at the first 60 minute time point, from the value for combined sugars just done above. This represents the *separate value* for sucrose content.

Appendix B: Table of wine sweetness, suggested dilution factors, and volumes to pipette (see step 4 of the Procedures)

Wine type:	Dry	Medium Dry	Medium	Sweet
Residual Sugar:	up to 4 g/l	up to 12 g/l	up to 45 g/l	over 45 g/l
Dilution Factor: (step 4 of assay)	none	5	25	50 (100 if over 100 g/L)
Volume of pH'd wine sample to take:	---	1.0 mL	0.40 mL	0.20 mL (0.10 mL)
Volume of diluent to add:	---	4.0 mL	9.6 mL	9.8 mL (9.9 mL)

Wine type designations and corresponding residual sugar values are taken from EU regulation 753/2002