Vinmetrica Residual Reducing Sugar User Manual

Caution: The Residual Reducing Sugar (RRS) kit contains corrosive and caustic reagents. For your health and safety, please pay close attention to proper use and disposal of these reagents as stated in this manual!

Materials provided in the kit:

1. Clarifying Powder (PVPP) (PN: RRS-4)
2. Copper Sulfate Solution (PN: RRS-5)
3. RRS Binding Solution (Rochelle Salt) (PN: RRS-6)
4. RRS Titrant (0.20 M sodium thiosulfate) (PN: RRS-7)
5. RRS Developer Solution (KI) (PN: RRS-8)
6. RRS (1+5) Sulfuric Acid Solution (PN: RRS-9)
7. Starch Indicator Solution (PN: RRS-10)
8. Reaction flasks (125 mL Erlenmeyers) (2) (PN: RRS-12)
9. 10.00 mL Volumetric pipette (PN: RRS-11)
10. Pipetting safety bulb (PN: SC-300-16)
11. Transfer (“Squeeze-bulb”) pipettes (3) (PN: SC-100-5)
12. 10 mL Sampling pipette (PN: SC-50-8)
13. 5 mL Sampling pipette (PN: SC-200-6)
14. 15 ml Conical tubes (5) (PN: SC-50-7)
15. Filtration kit: 5 mL syringe (PN: SC-100-6), 25 mm filter holder (PN: RRS-13), 25 mm filters (15 pieces, PN: RRS-14)

Things you will need:

1. Distilled water (DI water), which can be found at most grocery stores.
2. 10 mL burette; available from Vinmetrica (PN: SC-300-7-10)
3. Burette Clamp; available from Vinmetrica (PN: SC-300-6)
4. Lab Support Stand; available from Vinmetrica (PN: SC-300-3)
5. Baking soda (sodium bicarbonate), found in stores, to neutralize solutions for safe disposal.
6. (Optional) Additional reaction flasks (for running more than two tests at one time). Available in sets of two (RRS-12) or in sets of five (RRS-12-5).
7. (Optional) Additional 15 ml conical tube(s) (per test)
8. (Optional) Rinse bottle; available from Vinmetrica (PN: SC-100-17)
9. (Optional) laboratory centrifuge, or filter funnel with lab-grade #1 filter paper
10. (Optional) Burette detergent (PN: SC-300-12)

Why Test for Residual Sugar?

Residual sugar (RS) refers to any significant concentration of sugar that is contained in wine, beer or cider at the end of fermentation. Winemakers and brewers are typically most interested in knowing the concentration of the fermentable hexoses glucose and fructose, the main reducing sugars. These determine the level of sweetness of the finished product. At residual sugar levels around 2 g/L (0.2%) or higher, an alcoholic beverage can spontaneously restart fermentation unless it has been properly stabilized.

How it works:

The Residual Reducing Sugar (RRS) test, also known as the Rebelein or Gold Coast Method, determines the amount of residual sugar through the use of excess copper (Cu^{+2}). Reducing sugars are oxidized, reducing the copper. Remaining Cu^{+2} then is converted to iodine, which is titrated with sodium thiosulfate to determine the quantity of residual reducing sugars.

Because of this chemistry, non-reducing sugars are not detected. In general, non-reducing sugars are present in minor amounts in wine and fermented products and can be neglected. In some cases sucrose may be present in some wines and other beverages; measuring sucrose requires a hydrolysis step. (See below under Assay Notes, and in Appendix C).

Assay Notes:

- **Accurate pipetting and titrating are essential!**
  - See Appendix A and B for tips on using your 10.0 mL pipette and burette.
  - Make sure the 10.00 mL pipette and burette are clean. If droplets remain on the inside while delivering the Copper Sulfate Solution from the pipette, or in delivering the RRS Titrant from the burette, the glassware is dirty and there is a risk of irreproducible results and error. Rinse twice with DI water. If droplets still adhere, clean the burette or pipette by repeatedly passing through a hot ~1% solution of Burette detergent (PN: SC-300-12) or similar lab detergent to clean them, followed by several rinses of DI water, until the pipette drains without leaving droplets on the inside.
- **It is highly recommended to label all pipettes with tape or indelible ink for each respective solution before starting this assay to avoid cross-contamination or other possible errors.**
- You may have to dilute your sample if it is sweet, or take a larger volume if it is dry. This assay will only be accurate if the sample being measured has less than 28 g/L (2.8%) of residual reducing sugars. Sweet wines frequently have levels higher than this. Likewise, a dry wine sample may require a larger volume for best accuracy. **See Appendix D** for more information.
- This method does not measure sucrose unless it has first been hydrolyzed into its components glucose and fructose. See Appendix C.
- When the tests are completed, it is recommended to have at least 5 g of baking soda **per test** in order to neutralize the end solutions safely.

Procedures

The test consists of 5 steps. *Sample preparation* may be necessary to clarify wines. The *Reaction step* mixes the prepared wine with reagents and heat to react the wine's reducing sugars. After the reaction, you perform a *Titration*. From this you can do the *Calculation*. Finally, you'll be *finishing up* with a safe disposal step. **Note: keep sodium bicarbonate (baking soda) handy to neutralize a spill. Do not pipette by mouth! Wear gloves and eye protection!**

Sample preparation: Clarify the wine sample if needed.

1. If the wine contains significant precipitates, remove them by filtering, centrifuging or decanting.
2. Red wines need to be decolorized for accurate results.
   a) Place 0.4 g of Clarifying Powder into a 15 mL conical tube; this amount should fill the conical tube to just under the 2.0 mL mark on the tube.
   b) Add 6 mL of red wine sample. Cap the tube.
   c) Shake intermittently (or constantly) for 5 minutes.
   d) Allow the sample to settle for a few minutes, then separate the liquid from the solids using the filtration apparatus of the kit (see Appendix E). You can also centrifuge or settle the sample.
   e) If you centrifuge or settle your sample, the tube will have a clear to slight pink liquid supernatant at the top, and a settled precipitate on the bottom. Transfer the supernatant into a separate container with a transfer ("Squeeze-bulb") pipette. This prevents the sample from getting mixed up and cloudy when pipetting into the reaction flask.

Reaction step: As per below, place the Copper Sulfate and Binding solutions in the reaction flask. Add samples and heat. Include a Blank! Let the samples cool before proceeding.

1. Make a water bath at 95-100°C (200-210°F, or just boiling) for the heating phase in step 9 below.
2. Rinse reaction flasks twice with distilled water. Place inverted on a lint-free surface and allow to drain completely. Examine the flasks carefully to be sure they are clean before using them in the assay. They do not have to be dry.
3. With the 10.00 mL volumetric pipette and pipetting bulb, introduce exactly 10.00 mL of the Copper Sulfate solution into the bottom of a reaction flask. **Do not pipette by mouth!** It is important that this be done in a reproducible manner each time. **Accurate pipetting in this step is essential** to success and accuracy. See Appendix A for explanations of pipetting techniques.
4. With the 5 mL plastic sampling pipette and pipetting bulb, add 5 mL of Binding solution to each reaction flask. **Do not pipette by mouth**! Prepare as many reaction flasks as you have samples.
5. If your residual sugar level is expected to be above 28 g/L, dilute the sample quantitatively

with DI water to bring the approximate level to below 28 g/L. See *Appendix D for more information.* After proper dilution of samples, proceed to step 6.

6. Add the appropriate volume of prepared sample as directed in Appendix D. Usually 2.0 mL of sample, either diluted or not, is added accurately with a 5 mL sampling pipette to each respective reaction flask. For a dry wine, a larger volume, up to 16 mL, may be best for higher accuracy. See Appendix A for explanations of pipetting techniques.
7. Label the flasks to indicate the respective samples.
8. Include a blank, in which the sample is just pure DI water (2 mL).
9. Place all flasks in the hot water bath for exactly 2 minutes and 30 seconds. Immediately take the heated samples out of the water bath and let them cool to room temperature in air or in a cold water bath.

Note: Samples with high residual sugar content can show reddish residue from the reaction of the Copper sulfate. If a sample is extremely bright red after heating, all the copper has reacted and the result will be inaccurate. Repeat the Reaction step on a diluted sample (see step 5 above).

Titration: After the flasks have cooled, the titration step can begin. As per below, add starch, sulfuric acid, and RS Developer. Then, **immediately** titrate with the sodium thiosulfate (RRS Titrant) to the endpoint of each sample. NOTE: Delaying the titration can cause loss of the iodine that is generated by the Developer, causing increased error.

1. Fill the 10 mL burette with RS Titrant to the 0.0 mark (or write down the starting volume value, but always start no lower than the 1.0 mL mark so you don't run past the 10.0 mL mark when titrating).
2. For each sample and blank, add 10 mL of Sulfuric acid solution, 2 mL RS Developer, and 2 mL Starch solution; swirl briefly. When measuring the sulfuric acid, use the 10 ml plastic pipette and the pipetting bulb. **Do not pipette by mouth!** For the Developer and Starch, use separate squeeze bulb pipettes, and note the markings to be sure you deliver at least 2 mL. A dark green-black color will form (see Figure 1 below).

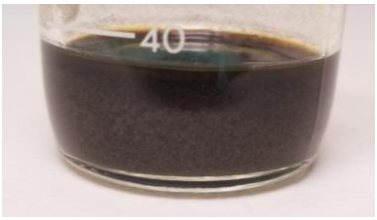

Fig. 1: Color of the reaction before titration starts

3. **Immediately** titrate the flask dropwise, swirling continuously, until the color changes from a dark green-brown to a grey-lavender color (Figure 2). This is very near the endpoint.

Fig. 2: The color of the reaction when titration is almost done

4. Carefully continue to titrate dropwise, swirling for approximately 5-10 seconds after introducing each drop. For best accuracy, try to hit this endpoint with incremental additions of half a drop. The titration is finished when the light grey-lavender color turns completely cream-colored/white (see Figure 3 below).

Fig. 3: Color of the reaction through its final drops of titration

Be sure to set aside spent reactions for proper disposal (see "Finishing up" below).

5. Note the final volume on the burette. Subtract the initial volume to get V, the titration volume used.

Table 1 below gives approximate titration volumes you should expect. This is just a guide. Your results may differ slightly.

Table 1. Approximate expected titration volumes (2 mL sample)

Sample RS g/L:	0 (Blank)	4	10	15	20
Volume of RS Titrant (mL):	8.4	7.3	5.6	4.2	2.8

Calculation: Call your blank titration volume V_b, your sample titrations V_a; then the residual reducing sugar level is calculated as[1]

$$\textbf{RRS, g/L} = (V_b - V_a) \times 3.63 \quad \textbf{(Eq.1)}$$

Example: A blank titration was 8.45 mL. A 2 mL wine sample gave a titration of 5.06 mL. So RS = (8.45 – 5.06) x 3.63 = 12.3 g/L. This is equivalent to 1.23% residual sugar.

Dilution Notes:

- As illustrated in Appendix D, if the sample is expected to be sweet (over 28 g/L), dilute it to be less than 28; usually a 1:2 dilution, accomplished by taking 5 mL of sample and adding 5 mL of DI water, is adequate. A very sweet dessert wine may require a 1:4 or 1:8 dilution.
- Also as shown in Appendix D, if the wine is expected to be dry, with residual sugar less than 4 g/L, you can try taking a larger volume for assay - up to 16 mL instead of 2.0 mL. Adjust calculations; see below.

[1] The equation can also be written as **RRS, g/L = (V_b – V_a) x M / 0.0551**, where M is the molarity of the thiosulfate in the RS titrant.

- If you have diluted your sample prior to the assay, multiply Eq.1 (above) in your calculations by the dilution factor to get the final result. For example, if you diluted a sample with an equal volume of water prior to taking a 2 mL sample, then the dilution factor is 2. If you have taken a larger volume for a dry wine, divide Eq. 1 by the ratio of the larger volume to 2 mL, e.g., if you took a 4.0 mL sample, then divide Eq. 1's result by (4.0 / 2.0) = 2.

Finishing Up: Safely dispose spent reactions and rinse.

1. **Carry out the following steps in a well-ventilated area. Use gloves and safety glasses.**
2. Pour the spent contents of the reaction flask into a glass or polyethylene plastic waste container; rinse the Reaction flask with about 20 mL of tap water and add this to the container. If you have multiple spent reactions, add them together into the container.
3. Add at least 5 g of baking soda **per test** to neutralize the contents of the container.
4. This can be sewered safely in most cases; check with local regulations.
5. Rinse all glassware with distilled water and let air dry.

WARRANTIES AND LIABILITIES

1. The materials provided in the kit, as described on page 1 above, ("Materials") are warranted as follows: All non-reagent components are warranted against defects in workmanship for 1 year 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.

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Appendix A: Pipetting accurately

ALWAYS use eye protection and gloves (latex or nitrile) when using glassware and chemical reagents. NEVER pipette by mouth! Use the provided pipetting safety bulb as described below!

It is important to develop a consistent, reproducible procedure for sampling and distributing reagents and samples. There are 2 steps in the RS assay that require high precision pipetting for accuracy.

1. Pipetting the Copper Sulfate. You will use your 10 mL volumetric pipette to dispense the Copper sulfate solution into the Reaction flasks. It is critical that the volume delivered be 10.00 ± 0.05 mL.

a. Be sure the pipette is clean and dry at the beginning of the session.
b. Holding the pipette in one hand, the bulb in the other, immerse the pipette into the Copper sulfate bottle so the tip is about 1 inch below the surface.
c. DO NOT Pipette by Mouth! Use your pipetting bulb to suck the blue-colored liquid into the pipette slowly until it rises to above the mark.
d. In one quick movement, remove the bulb and place your index figure over the end of the pipette to control the release of the liquid. (Illustration 1)
e. Allow the liquid to flow back into the Copper sulfate bottle until the meniscus touches the mark. (Illustration 2)
f. Touch both sides of the pipette tip to the inside lip of the Copper sulfate bottle to drain off any droplet that may be clinging to the outside of the pipette tip.
g. Place the pipette tip against the inside wall of the reaction flask you are filling. (Illustration 3 shows use of a 100 mL reaction bottle, a suitable alternative to the 125 mL reaction flasks)
h. Allow the liquid to drain completely. DO NOT blow out the liquid!
i. At the end of the draining, keep the tip lightly held against the wall of the flask and twist/rotate the pipette back and forth five times to complete the delivery. (Illustration 4)

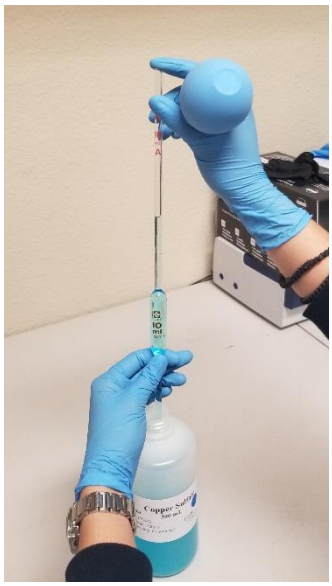

Illustration 1: step d. controlling the speed of drain from the pipette.

Illustration 2: step e. Where the meniscus touches the mark.

Illustration 3: step h. Allowing the copper sulfate to drain from the pipette.

Illustration 4: step i. Rolling the pipette to complete delivery of the copper sulfate.

j. Do not rinse the pipette between successive fillings of reaction flasks; rather, place the pipette carefully in a clean glass container tip down, or lay it on its side in a manner to prevent the tip from touching anything. When all reaction flasks have been filled, carefully rinse the pipette into the waste container used for spent reactions (see "Finishing Up" on page 6). Rinse the pipette twice with distilled water and allow to drain.

2. Pipetting the sample. It is important to deliver this volume accurately (2.0 ± 0.1 mL). We recommend using the 5 mL plastic sampling pipette.

a. Be sure the pipette is clean and dry at the beginning of the session.
b. Holding the pipette in one hand, the bulb in the other, immerse the pipette into the filtered or centrifuged sample supernatant to as low as possible without disturbing any settled precipitate.
c. Use your pipetting bulb as shown to suck the clear liquid into the pipette slowly until it rises to just above the 3.0 mL mark.
d. In one quick movement, remove the bulb and place your index figure over the end of the pipette to control the release of the liquid.
e. Allow the liquid to flow back into the container of the sample until the meniscus touches the 3.0 mL mark.
f. Allow the liquid to drain completely into the sample's respective reaction flask, touching off the tip to get complete draining. A small amount of liquid remains in the tip.
g. Rinse out the sampling pipette with distilled water before pipetting a new sample.

Appendix B: How to use your burette

ALWAYS use eye protection and latex or nitrile gloves when using glassware and chemicals.

To get the most accurate results when titrating, there are a few things to keep in mind.

1. Filling
   a. Each day of use you should first rinse the burette with a few milliliters of Titrant to remove any excess water or contaminants that may remain from a previous titration. Allow the burette to drain completely. Discard this rinse.
   b. When filling the burette, make sure the Titrant has completely filled the bottom of the burette, including within its tip. Sometimes bubbles can be trapped in the tip of the burette; these can usually be dislodged by opening and closing the stopcock while the burette is hovering over a waste container.
   c. Make sure there are not any large bubbles in the burette after filling. If there are, wrap the top of the burette with some plastic wrap (or Parafilm if you have it) and make sure the stopcock is in the closed position. Then take the burette out of its clamp and hold the wrapped end tightly with your thumb. Rotate and invert the burette to allow the bubbles to move out of the column of Titrant.
   d. Once all bubbles have been displaced, replace the burette in its clamp and set the level to 0.0 or slightly below. Make sure that no drop is hanging from the burette's tip, as this will contribute to error. Now you are ready to titrate.

2. Reading:
   a. A simple trick makes accurate reading easy. Draw a 1-inch black band down the center of a thick sheet of white paper, note card, or the back of a business card (Illustration 5).
   b. When taking a measurement, hold the paper about an inch behind the burette with the black band about a half an inch below the meniscus (Illustration 6). This provides a clear view of the bottom of the meniscus which helps make a precise, consistent measurement.
   c. You should be able to read to a resolution of half a gradation, or 0.025 mL on your 10 mL burette; with a little practice, you can read to within a fifth of a gradation (0.01 mL).

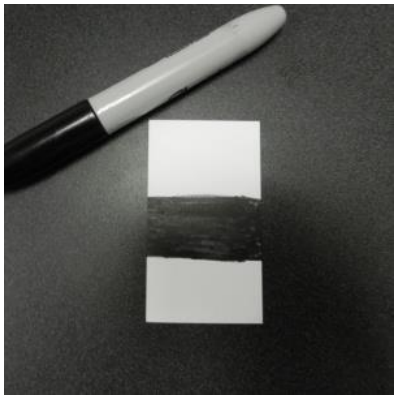

Illustration 5. draw a 1-inch black band on card paper. This card will assist you in reading the burette accurately.

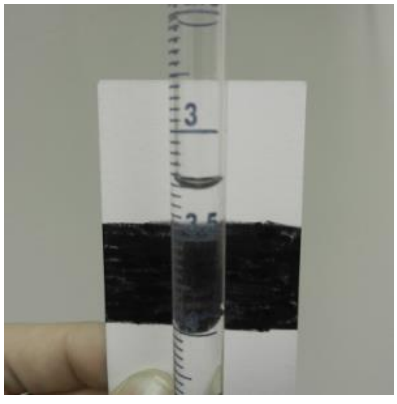

Illustration 6. Reading the meniscus of the liquid in the burette. In this picture the value is 3.26 mL.

3. Titrating:
    a. Before beginning the titration, read the starting titration value. Record this value in your notebook.
    b. Rinse down the inner rim and walls of the flask with a few mL of water as directed. Add the Acid, RRS Developer and Starch to the flask as instructed. Immediately begin titrating slowly, keeping the flask contents swirling to enhance the titration reaction. As the endpoint nears, the solution's green/black color will give way to light purple/lavender color (see Figures 1 and 2 on page 4).
    c. At this point, rinse the sides of the flask once more with a few mL of water Now add the Titrant one drop at a time with swirling of the flask for a few seconds in between each drop, to give the reaction enough time to respond.
    d. Try to hit the endpoint with half-drop increments for best accuracy. A half drop can be gently shaken off the burette tip, or can be touched off to a point on the inside of the Reaction flask with swirling to mix. The endpoint is reached when the lavender color changes suddenly to a light cream white. (see Figure 3, page 5).
    e. When you have reached the endpoint, immediately read and record the final titration value. The volume of Titrant used is the difference between the final value and the starting value.
4. Cleaning:
    a. Always rinse your burette out with distilled water at the end of the day. Store the burette inverted and/or covered with a cap to prevent particles and contaminants from entering.
    b. If the burette becomes dirty, you will see that droplets of Titrant remain clinging to the walls of the burette. Since these droplets are supposed to be part of the titration, your results will be inaccurate. In that case it is necessary to clean the burette.
    c. Try filling the burette with a moderately hot detergent solution, like 1% Alconox or other glassware cleaning solution (available from Vinmetrica, PN: SC-300-12). Allow it to soak for 30 minutes in the solution. Rinse several times with distilled water. Repeat as necessary until water drains smoothly without drops remaining behind.
5. You can also check out this website for more burette info:
   http://www.titrations.info/pipette-burette

Appendix C: Hydrolysis of sucrose in samples

ALWAYS use eye protection and gloves (latex or nitrile) when using glassware and chemical reagents. NEVER pipette by mouth! Be sure there is adequate ventilation.

During fermentation virtually all sucrose that may have been initially present is converted by the yeast into fermentable reducing sugars. So generally it is not necessary to analyze wines, beers and ciders for sucrose. In certain circumstances sucrose may be present. An example would be if sucrose has been added post-fermentation. In such cases, the following acid hydrolysis method will convert sucrose into glucose and fructose, making them assayable by the reducing sugar procedure.

Contact Vinmetrica if there are any questions. Note: At this time, Vinmetrica does not provide the reagents or equipment (except the reaction flask and the 25 mL sampling pipette) listed below.

Equipment
60°C hot water bath
Cold or ice water bath
125 mL Erlenmeyer flask
50 mL volumetric flask or 50 to 100 mL graduated cylinder
25 mL sampling pipette or graduated cylinder

Reagents
Ammonium hydroxide solution 1 + 1.5
HCl solution 1+1

Procedure
1. Measure 25 mL wine or other sample into a 125 mL flask.
2. Add 10 mL 1+1 HCl.
3. Place flask in 60°C water bath for 15 minutes. Swirl occasionally.
4. Cool flask in cold water bath to room temperature.
5. **Slowly** add 10 mL of ammonium hydroxide solution 1 + 1.5
6. Adjust total volume to 50 mL with DI water in volumetric flask or graduated cylinder
7. Mix thoroughly.

This hydrolyzed solution can now be analyzed in the Residual Reducing Sugar procedure. Note that you have already diluted the original sample by 2, so this should be factored in when performing the final calculation of result.

Appendix D: Table of wine sweetness, suggested dilution factors, and volumes to pipette (see step 6 of the Reaction Step under Procedures)

Wine type:	**Dry**	**Medium Dry**	**Medium**	**Sweet**	**Very Sweet**
Residual Sugar:	up to 4 g/l	5 to 12 g/l	13 to 45 g/l	45 to 100 g/L	over 100 g/L
Dilution Factor:	1 (undiluted)	1 (undiluted)	2 (e.g. dilute 2 mL with 2 mL water)	4 (e.g. dilute 1 mL with 3 mL water)	8 (e.g. dilute 1 mL with 7 mL water)
Volume of prepared wine sample to take:	4.0 –16.0 mL	2.0 mL	2.0 mL	2.0 mL	2.0 mL

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

Appendix E: Using the Filtration kit

1. Open the 25 mm filter holder by turning the two halves counterclockwise to each other. Make sure the silicone O-ring stays seated in the inlet side.
2. Place a 25 mm filter disk on the flat surface of the outlet side.
3. Reassemble the two halves and hand-tighten firmly. Be sure the filter and O-ring stay in place while tightening.
4. Use the syringe to pick up 5 mL of the decolorized wine mixture, leaving as much of the settled precipitate behind as possible.
5. Insert the syringe firmly into the receiver on the inlet
6. Place the end of the filter outlet in a small tube or similar vessel
7. Push the syringe plunger steadily and firmly to get the filtered wine sample into the tube. Do not apply excessive pressure!
8. The filtered solution should be clear. If there is cloudiness, disassemble the filter holder to check the integrity of the O-ring and the filter disk. Re-position or replace the filter if needed and repeat.
9. A filter in its holder may be back-flushed, or gently disassembled and washed, with water, for re-use. Discard the filter disk after three uses.