Kit Item Check-List:

Y402 — (2x 125 mL flasks)
MT142 — (2x 2-hole #5 stoppers)
MT141 — (3-piece glass tubing set)
D1700S — (20° of 3/16" silicon tubing)
R316 — (24" of clear 1/8" tubing)
FE383 — (1 pump, includes ¼" flow regulator valve)
HS70 — (2 "quick-disconnect" assemblies)
MT102A — (1 squeeze bulb for pipettes)
MT118 — (1 10 mL syringe)
MT641 — (1 10 mL pipette)
MT642 — (1 20 mL pipette)

Reagents:

MT107A — (Hydrogen Peroxide 3%)
MT108A — (Phosphoric Acid 25%)
MT110A — (Sodium Hydroxide 0.01N)
MT120A — (HCL 0.01N)
MT111 — (SO₂ indicator)

Congratulations on your purchase of MoreWine!’s Economy Aeration-Oxidation system! Before going any further, please open all the packaging for your kit and lay out the parts on a table so you can clearly see what you’ve got. Please double check the contents of your shipment against the list of parts shown above. If anything is missing or damaged please contact our Customer Service Department immediately.

The next step is to assemble the system. Simply assemble the system so that it matches what you see in the photo above. Here are a few helpful tips:

• The MT140 comes with two stoppers and three glass tubes (one short and two long). The tubes will need to be inserted into the holes in the stoppers so that one stopper has both a short and a long tube in each of its holes, and the other stopper has only a long tube in one of its holes (the other hole is left open). The pointed tips should face into the flask. Adjust the height of each of the long tubes so that the pointed tip sits about 1/8” above the bottom of the flask when the stopper is firmly closed. For the short tube, the exact height placement is not critical, just make sure the end passes through the stopper completely to ensure a firm hold.

Helpful Tip: Wetting the glass with a small amount of water will help the tubing slide through the stopper much more easily.

• Each flask will have a slightly different opening size and as a result its stopper will end up at a different height when sealed. For this reason we recommend designating one of the flasks as the “wine sample” flask (an permanent marker will allow you to label the flask “Wine Sample”). The fact that the wine sample flask’s stopper has two tubes in it and the other flask only has one allows us to easily tell them apart. This way when we adjust the long tubes to have our desired 1/8” gap from the bottom of the flask they will always be at the ideal height.

• There is nothing special that you have to do to insert the R316 tubing into the D1700S tubing, and this connection does not require a hose clamp in order to stay secure.

• Make sure all inserted connections are pushed in 3/4 to 1”.

• The HS70 plastic Quick Connects are designed to allow you to easily remove each of the stopper/tube assemblies for cleaning.

• We have supplied you with an excess of tubing for all portions of the system. We recommend cutting it roughly to the sizes shown in the photograph. Please trim the tubing to fit appropriately and either keep or discard the excess. The exact measurements will not have any impact on the result of the test.
Running the Test - Overview

In brief, the procedure involves adding an acid and then agitating a wine sample so that the Free SO₂ gets driven off as a gas. By design, the only way for the gas to exit the system is to be passed through the second flask, which is filled with Hydrogen Peroxide and a colored indicator. As the gas passes through the solution, the Hydrogen Peroxide actually converts the SO₂ to Sulfuric Acid. As a result of the change in pH from making the acid, the indicator changes color from gray-green to purple. Upon reaching the end-point of the test, you then neutralize the Sulfuric Acid you made with a base, Sodium Hydroxide (NaOH). Since all the acid was made from the SO₂ in your wine sample, a simple calculation can relate the amount of NaOH that was needed to neutralize the acid to the amount of SO₂ that was originally present in the wine sample. We strongly recommend that you read through the entire set of directions before performing the test. So, let’s begin:

Equipment needed

- MT140 (this contains all of the basic equipment needed to run the test*)

*Note: If you will be frequently running multiple tests during a single session, then you may want to think about investing in a titration stand with a self-zeroing burette (MT150).

Reagents involved:

- MT107A (3% Hydrogen Peroxide, same as what you buy at the drugstore)
- MT111 (SO₂ indicator)
- MT110A (0.01N NaOH — Sodium Hydroxide)
- MT108A (25% Phosphoric Acid)
- MT120A (0.01N HCl — Hydrochloric Acid)

Setting-up the test

Preparing the reaction solution:

1) Using the 10 mL pipette and the red pipette bulb, transfer 10 mL of 3% Hydrogen Peroxide into the flask whose stopper has the single glass tube and open hole at the top. This will be your reaction vessel for the test.

2) Next add 6 drops of the SO₂ indicator into the 3% Hydrogen Peroxide you just placed in the flask and gently swirl to mix the two together. Now add 40 mL of distilled water and bring the liquid up to the 50 mL mark on the flask. Important: It’s important to only use distilled water here; tap and mineral water contain minerals that can throw the test off.

3) Now we need to calibrate the Hydrogen Peroxide solution to prepare it for the test. The SO₂ indicator solution works by changing color based on the pH of the solution it is in – bright magenta when the solution is acidic and bright green when the solution is basic. When the indicator is in a pH neutral environment it will be a grey/grey-green color. There is a VERY narrow range of pH where the indicator will be grey, even a very small shift in pH to either acidic or basic and the indicator will change color. Remember that at the end of the test you’ll be adding NaOH to the then magenta solution (magenta because you made it acidic when you created the sulfuric acid) until you get it back to gray-green. Because the result of the test is based on how much NaOH this takes, it is important to have the correct starting point and the correct end point.

When using store-bought hydrogen peroxide it is common for the solution to turn magenta when the indicator is added, as the peroxide can be slightly acidic. As you add the distilled water, the solution will become more pH neutral and the color usually shifts to a watery gray and then to a clear green color.

Note: Depending on the water that you use for this step it is possible that the solution will stay pink. If this is the case please skip ahead to Step 4, and add the Sodium Hydroxide until the solution reaches the gray-green color described there.

Ideally, at the start of the test we want the indicator at the exact point where the grey turns to green. However, when we added our distilled water we have no way to know how far we have gone into the green range, since
once the indicator turns green it will stay the same color of green no matter how much more basic the solution becomes (the same is true for the magenta/acidic side of things). Fortunately, there is an easy way to get us back to the grey range. Using the syringe, draw up about 4 mL of the 0.01HCl. Begin to add it carefully at a rate of 1 drop at a time into the Hydrogen Peroxide solution while continuously swirling the flask. Eventually the solution will turn from a clear green to a watery grey color. At this stage add one more drop and swirl the flask. The solution should turn pink (if the solution stays grey, go ahead and add another drop of the HCL until the pink color is reached). Now we have a clear picture of where on the pH continuum our Hydrogen Peroxide solution is – one drop into the pink range. You’re ready to move on and finish prepping the peroxide solution.

4) Return any remaining HCL in the syringe back into the bottle. Rinse your syringe with water and draw up 2 mLs on your 0.01 NaOH. Add 1–3 drops of NaOH to our pink solution (swirl slowly between each drop) until you get back to the gray-green color. Go slowly so that you don’t overshoot the color you are looking for or you will have to discard the peroxide and start over. That’s it, the reaction solution is ready to go.

5) Install the stopper on to the flask so it fits firmly and makes a nice seal. Make sure the tip of the impinger (long glass tube) is completely submerged into the colored Hydrogen Peroxide solution.

This portion of the preparation is now complete.

**Preparing the wine sample:**

1) Using the 20 mL volumetric pipette and the red pipette bulb, transfer 20 mL of wine sample into the other flask (whose stopper has the two pieces of glass in it). Attach the stopper. Make sure the tip of the impinger (long glass tube) is completely submerged into the wine sample.

2) Using the 10 mL serological pipette and the red, pipette bulb, transfer 10 mL of 25% Phosphoric Acid into the flask containing the wine sample.

3) Quickly attach the bubbler stopper to the flask, making sure both stoppers are snugly attached.*

*Note: Upon addition of the Phosphoric Acid, the Free SO\textsubscript{2} will immediately begin to volatize out of the wine sample. In order to maintain the accuracy of the test, it is important to minimize the amount of gas that might escapes once the acid has been added to the sample; so you will want to be quick about getting the flask sealed once you’ve added the acid.

**Running the Test**

Once you have both the impinger and the wine sample prepared it is time to go ahead and run the test.

1) Turn on the pump (plug it in).

2) Slowly turn the regulator valve counter-clockwise (it’s basically a threaded screw) until the wine sample, as well as the Hydrogen Peroxide solution inside the other flask will begin to bubble. Open the valve 2–3 turns until the wine sample bubbles at a slow but steady rate.

3) Allow the test to run for 10–15 minutes. As mentioned above, if there is Free SO\textsubscript{2} present the sample will change color from gray-green to magenta. A lack of a color change indicates one of two things: A) (most common) There is no SO\textsubscript{2} in the sample; or B) Your Hydrogen Peroxide is old and has gone bad. Once opened the peroxide has about a 2–3 month shelf life and you will be able to tell that it is spent because it will no longer turn the indicator magenta right away.

4) Once the test has gone to completion, turn off the pump. Loosen the bung on the flask with the colored 3% Hydrogen Peroxide solution and carefully remove the impinger tube to free up the flask. You will perform the next step using this flask.

5) Now it is time to add the NaOH. Draw 10 mL of NaOH into the syringe that was included with the kit (10 mLs allows you note the starting volume accurately). Begin slowly adding the NaOH one drop at a time* into the Hydrogen Peroxide solution while swirling the flask to mix it thoroughly between drops.
*Note: Do not get carried away and begin adding several drops at a time — the color will change very quickly from magenta to gray-green, usually within 1-3 drops, and adding too much at once can cause you to overshoot the endpoint by an unknown amount. Once the solution in the impinger flask is the same color it was before you started the test, make a note of the volume remaining in the syringe.

6) The difference between the starting volume in the syringe and the final volume in the syringe is the amount of NaOH you used. This is the value you’ll use to calculate the amount of SO₂ that was originally present, according to the formula below.

Free SO₂ (ppm) = mL NaOH used x 16

Example: If 1.7mL NaOH was needed to reach the endpoint of the test, then:

1.7mL x 16 = 27.2 ppm of Free SO₂ is present in the wine sample.

*Note: The above formula only holds if you are using 0.01N NaOH, which is the concentration we include with this kit and sell as a supply. Do not confuse this with 0.1N NaOH which is the strength used for TA testing. Using 0.1N for this test is not feasible because of the large difference in strength.

Important Information

- You will need to store the Hydrogen Peroxide tightly-sealed and in the refrigerator, but will need take it out and allow it to warm to room temperature before using it to run your tests. The strength of the Hydrogen Peroxide used in the procedure (3%) is more than adequate for the needs of the test, and it will remain effective around three months once opened.

- The NaOH should be stored sealed and in the fridge as well, and also returned to room temp prior to use. This concentration of NaOH should keep for 3–4 months.

- To avoid any loss of gas in the system (and therefore lower the accuracy of the results), make sure all of the connections are tight.

- We strongly recommend picking up a Laboratory Wash Bottle (MT644) for cleaning out the flasks and your pipets.

- If you will be running multiple tests at the same time, you may want to get a second 10 mL pipette (MT641). This way you can designate one to the Hydrogen Peroxide and the other to the Phosphoric Acid (we use a piece of masking tape to designate the pipette used for acids in our own lab). Having two separate pipettes for acids and bases will also decrease the likelihood of cross-contaminating your solutions. Keep in mind that any 25% Phosphoric acid left over on the pipet when you next use it for Hydrogen Peroxide will contaminate your bottle of peroxide and distort the results of the test.

  *Important:* While the small amount of Sulfuric Acid that you create during the test is neutralized at the end, the 25% Phosphoric Acid that you used to drive the SO₂ out of the wine sample is not consumed at any point. This means that it is still present in the spent sample at the end of the test. For the benefit of our environment, we request that you discard this sample in a plastic bucket and neutralize it with baking soda prior to putting it down the sink or toilet. You’ll be able to tell when it is safe to discard because the solution will turn green and stop foaming when you add baking soda if it is neutral.

- If at any point you believe that you have gotten some of the Phosphoric Acid on your skin, you should immediately stop what you’re doing and rub the area down with baking soda, which will neutralize the acid. Please note that a chemical burn does not set in immediately the way a heat burn does, but can be just as painful and damaging to tissue. Because you may not realize that you have exposed yourself to the chemical right away it is important to treat yourself with the baking soda immediately if you even suspect you have gotten some acid on your skin. You may also elect to wear latex or nitrile gloves while performing the test.

- You may find it helpful to watch our video demo of how to set up and run your test on our website at www.morewinemaking.com. Just type MT140 into the “Product Search” field and then click on the product to view the video demo.