

Malolactic Chromatography Testing Kit Instructions



This Kit Contains:

- One Gallon Wide Mouth Jar
- 100 Capillary Pipettes
- Chromatography Papers (25)
- Chromatography Solvent
- Malic Acid Standard (0.3%)
- Tartaric Acid Standard (0.3%)
- Lactic Acid Standard (0.3%)

What does this test do?

This test will detect and identify lactic, malic and tartaric acids in grape juice, must or wine.

How does it work?

Paper Chromatography uses simple filter paper, a special solvent, and an indicator to separate complex mixtures into individual components. In the case of MLF (malo-lactic fermentation) monitoring in wine, the procedure simply allows one to identify the presence of tartaric, malic and lactic acids in a qualitative (or semi-quantitative) manner. Through capillary action, the acids move up the filter paper with help from the solvent.

Each acid, with its unique molecular properties, moves a characteristic distance up the paper and, with the help of acid standards, you can readily identify the acids in the wine sample. A color indicator is used to change the acid spot to yellow distinguishing it from the blue/green background colour developed on the paper.

Malic acid will show as a large spot on the paper before MLF but as the bacteria fermentation progresses, the malic spot diminishes and the lactic acid spot increases in size. At some point the malic acid spot is difficult to distinguish, which indicates MLF is nearing completion. However, since the paper chromatography procedure can only detect down to 100 mg/L (100 ppm), there could still be trace amounts of malic acid in the wine. As little as 30 mg/L can cause bubbling in your bottled wine. Therefore, one should allow the fermentation to continue until no more characteristic MLF bubbles appear in the neck of the carboy. This may take another week or so.

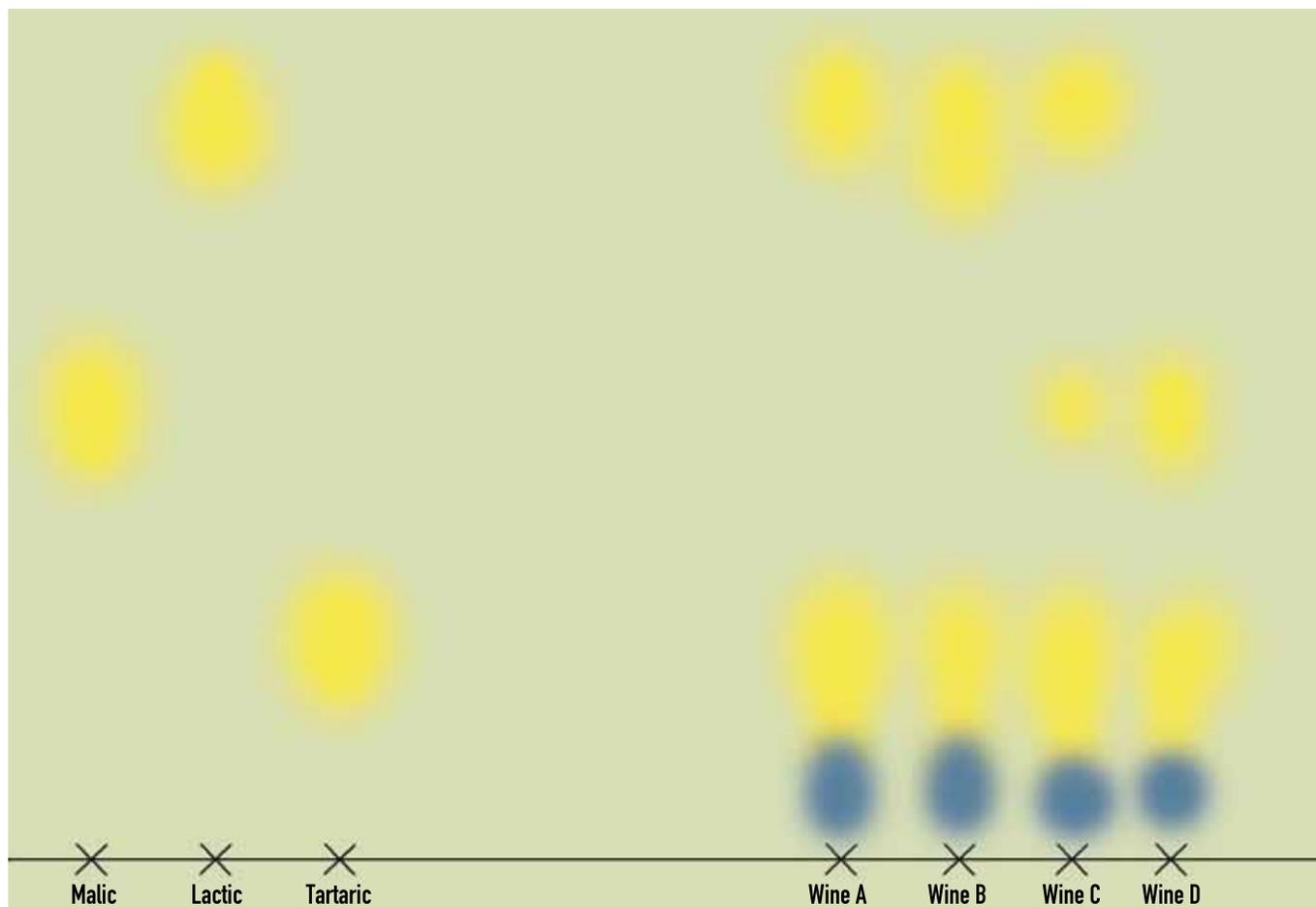
Preparation of Chromatogram

1. Draw a pencil line parallel to the long edge of the chromatography paper about an inch from the edge. Do not use pen as the ink will run with the solvent and be carried away. Mark an "X" on the left side of the line for each of the three acid standards. Do not place the marks less than 1 inch apart. Label the left X "Malic", the middle one "Lactic" and the right one "Tartaric". On the right side of the paper, mark an "X" for each of the wines you'll be testing. Label them so you'll know which is which.
2. Dip the clear end of a glass capillary pipette quickly into the Malic Acid Standard, allowing the solution to fill up ¼" to ½" of the tube. You may want to have a paper towel handy to blot some liquid on in case you draw too much solution. Place the tip of the pipette carefully at an angle on the X marked "Malic" and let the liquid flow into the paper. You're aiming to make a spot on the paper about ¼" in diameter. If you're having trouble getting the

liquid to flow out, try changing the angle or gently tapping the top of the pipette. Repeat this process for the Lactic and Tartaric standards on their respective marks. Be sure to use a fresh pipette for each standard to prevent cross contamination.

3. Now follow the same process described in step 2 to place your wine samples on their respective marks on the right side of the paper.
4. Once all your spots are placed, allow the chromatogram to dry for 30-60 minutes.
5. Take the one gallon wide-mouth jar, and carefully pour Chromatography Solvent in to cover the bottom $\frac{1}{4}$ to $\frac{1}{2}$ inch.
6. Staple the short ends of the chromatography paper together forming a cylinder. Do not overlap edges of the paper.
7. Lower the paper cylinder carefully into the jar and tightly seal the lid. Don't worry that the solvent isn't touching the spots you made on the paper; this is correct.
8. Place the sealed jar in a place where it won't be moved or disturbed until the solvent reaches the upper edge of the chromatogram. This may take anywhere from 4-8 hours. You can safely leave it overnight, even if the solvent reaches the upper edge of the paper.
9. Use tweezers to remove the cylinder from the jar and hang it in a well-ventilated place to dry.
IMPORTANT! Avoid breathing the solvent fumes for more than a few minutes at a time. The best place to hang your chromatograph is outside.
10. The solvent may be kept in the jar until it is all used up. Just make sure to seal the container tightly.
11. When completely dry the chromatograph will have a blue-green background with yellow spots of acids. Excess humidity will delay or prevent full development of blue-green background, however acid spots are easily visible. Drying in an air conditioned room or near a dehumidifier will complete color development. The presence of a particular acid can be confirmed by matching a spot above where you placed the wine sample with the height of one of the standard spots.

Sample Chromatograph (see how to read the Chromatograph on the following page):



Reading A Chromatograph:

The standard acid solutions on the left of the Chromatograph leave a yellow spot that is used as a reference. Compare the yellow spots left by your wine samples with the yellow reference spots left by the standard acid solutions to determine what acids are in your wine. Note that if the yellow spots have blurred together you may need to further separate your samples or use a smaller amount of each sample. Drying the Chromatograph in a warm, low humidity, environment will enhance the colors and make it easier to read.

Interpreting Wine Samples on Example Chromatogram:

- Wines A and B have a yellow spot indicating the presence of Lactic Acid, but no yellow spot indicating Malic Acid. This means that both wines A and B have completed malo-lactic fermentation (MLF).
- The fainter yellow spots on Wine C indicate the presence of both Malic and Lactic Acids. In the example, Wine C appears to be about halfway through MLF.
- The fact that Wine D shows a spot for Malic Acid but no spot for Lactic Acid means that Wine D has yet to enter an MLF.