

CHROMATOGRAPHY KIT WITH REAGENTS

Equipment

Gallon wide mouth jar with lid
Chromatography clips
Capillary tubes
Chromatography paper

Reagents

Chromatography solvent
Malic acid standard
Lactic acid standard
Tartaric acid standard

The malolactic chromatography kit with reagents is designed to detect the organic acids present in grape juice, must or wine. The technique, when applied to a wine sample, allows separation of tartaric, citric, malic, lactic and succinic acids. The technique can be used for monitoring malolactic fermentation in a wine by monitoring the formation of lactic acid as the malic acid disappears.

The test is simple to perform and results are visible and more reliable than any other commonly used procedure. The principle of paper chromatography is that as the solvent rises by capillary action (elution) through the paper the dissolved acids are attracted to both paper and solvent. Since each acid has a different affinity for the paper and the solvent, the different acids in the wine move different distances from the starting point and thus are separated from each other.

Procedure

1. Select a piece of chromatography paper, holding only the top corner with fingers.
2. Place the chromatography paper on a clean and dry bench area.
3. Draw a pencil line from side to side located about 2 cm from the bottom of the paper. Mark cross (x) lines at 2.5 cm intervals along the line.
4. Write the name of the respective standards and wines below each of the cross lines, using a pencil, NOT a ball point pen.
5. Raise the bottom of the paper above the bench by supporting it on a glass rod, pipette or pencil.
6. Using a capillary tube, keep the spots as small as possible, spot the samples exactly on the respective cross line a minimum of 4 times, allowing the spot to dry between applications. Spots may be dried quickly by applying a gentle stream of warm air from a hairdryer. Use a

ENARTIS USA

7795 Bell Road - Windsor, CA 95492
Tel: 707 838 6312 - Fax: 707 838 1765
www.enartis.com

The indications supplied are based on our current knowledge and experience, but do not relieve the user from adopting the necessary safety precautions.

Revision: January 2016

new capillary tube for each sample and standard.

7. When the spots are dry, curl the paper into a cylinder shape and clip the sides together at the top only (either staple or paperclip). Do not let page overlap.
8. Place sufficient chromatography solvent in the chromatography jar (or glass jar with lid) to provide a layer about 0.5 cm deep (covering the bottom of the jar).
9. Place the prepared paper into jar, spot side down, being careful not to touch the side of the jar.
10. Place the lid on the jar and leave until the solvent front has progressed up the paper about 20 cm in height (about 3 hours). Do not let the solvent front flow over the edge.
11. Remove the paper and allow to dry in a well ventilated area, away from any contaminating acid or alkaline vapors (about 6 to 8 hours). The paper will develop yellow spots on a green background.
12. Observe the position of each spot and by comparison with the position of the standard spots, identify the acids present in the tested wine sample.

Notes

If the developed chromatogram does not clearly show the green background with yellow spots, the winery environment has an acidic pH. To assist in the background color change, put the dry chromatogram in a basic environment. The easiest way is to open an ammonia based product near the chromatogram. In a volatile basic atmosphere the yellow acid spots should appear and you can observe the elution results.

Do not run the solvent front off the end of the paper. The acids will continue to elute and will be lost.

If the developed spots are too small or pale to see, introduce more sample during the spotting process. Insufficient sample volume can lead to errors in observation for malic acid depletion.

ENARTIS USA

7795 Bell Road - Windsor, CA 95492
Tel: 707 838 6312 - Fax: 707 838 1765
www.enartis.com

The indications supplied are based on our current knowledge and experience, but do not relieve the user from adopting the necessary safety precautions.

Revision: January 2016