Enzymatic kit for determination of L-Ascorbic acid

Principle:

In presence of L-Ascorbic acid and other substances, the reagent forms a purple colored complex (formazane).

$$\text{L-Ascorbate + other substances + MTT} \xrightarrow{\text{PMS}} \text{Dehydroascorbate + formazane + } H^+$$

For the specific determination of L-Ascorbic acid in wines it's necessary to measure the wine a second time after removing the L-Ascorbic acid with ascorbate oxidase (AAO).

$$\text{L-Ascorbate+ 1/2 } O_2 \xrightarrow{\text{AAO}} \text{Dehydroascorbate + } H_2O$$

The difference between the two results correspond to the quantity of L-Ascorbic acid.

The kit for 50 analyses includes:

Bottle	Composition	Quantity
A.	Buffer	100 ml
B.	MTT/PMS	16 ml
C.	AA0	2 tubes
D.	Diluent	20 ml
STD.	Standard	1 tube

Reagent preparation:

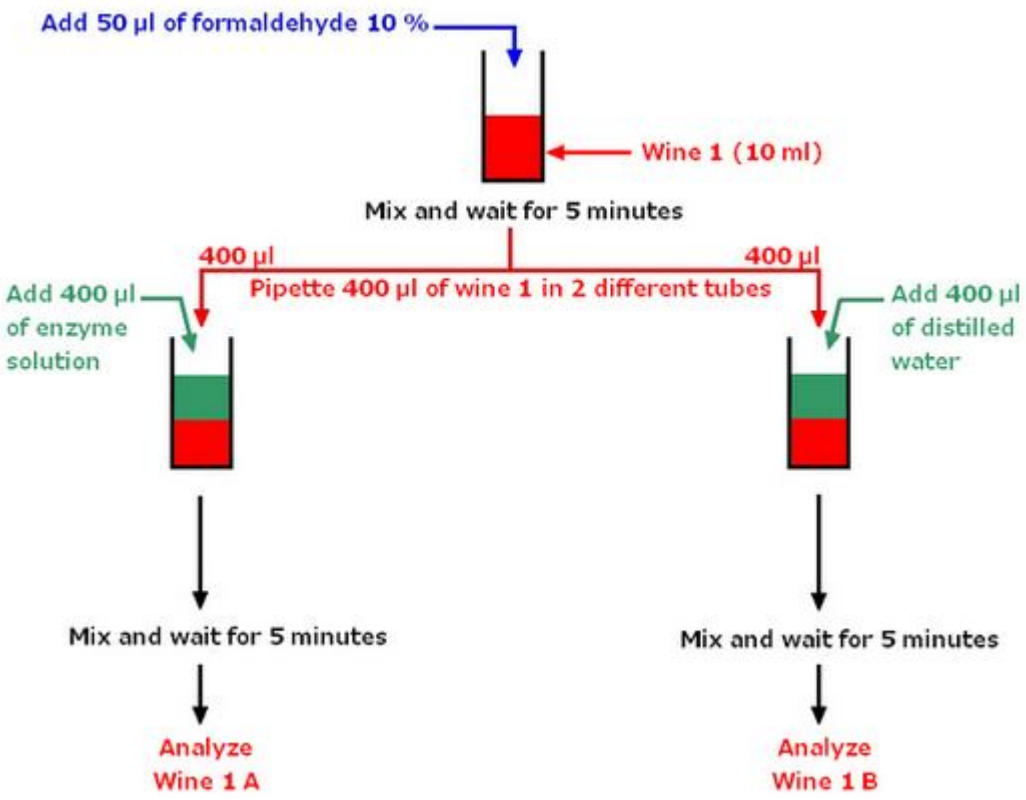
R1: is **Bottle A** and it's ready to use.

R2: is **Bottle B** and it's ready to use.

Enzyme solution is prepared by dissolving one **tube C** with 10 ml of **Bottle D**.
Stability of Enzyme solution: 3 months, if stored at 2 to 8°C.

Sample preparation:

Samples must be pre-treated with formaldehyde. Add 50 µl (one drop) of 10% formaldehyde solution into 10 ml of wine and mix .
After 5 minutes, pipette 400 µl of the pretreated sample into 2 different tubes or cups
(because the samples has to be analyzed 2 times).
In the first one add 400 µl of distillated water and in the second one add 400 µl of enzyme solution. Mix and wait 5 minutes.
The sample is ready to be analyzed and the difference between the two results correspond to the quantity of L-Ascorbic acid.



Preparation of L-Ascorbic acid control solution:

Weight 150 mg of **Standard tube** and dissolve in a 100 ml volumetric flask with distilled water.
This solution is stable for 1 week if stored in sealed bottle at 2 to 8°C.
Before analysis, dilute the stock solution by 10 in order to obtain a solution at 150 mg/l of L-ascorbic acid.
This solution corresponds to a control at 300 mg/l because the samples are diluted by 2 prior analysis.

Performances:

This test has been developed to determine the concentrations of L-Ascorbic acid in a measuring range from 0 to 300 mg/l.
If expected values are higher than 300 mg/l, samples should be diluted with distillated water and the result must be multiplied by the dilution factor.

Storage instructions and reagent stability :

The reagents are stable up to the expiry date, if stored at 2 to 8 °C. Contamination should be avoided. Do not freeze the reagents!

Warnings and precautions:

Do not swallow the reagents. Avoid contact with the skin and mucous membranes.
Take the necessary precautions for the use of laboratory reagents.

Sample analysis:

Wavelength: 578 nm.
Semi-micro cuvette: 1 cm light path (plastic or glass).
Temperature: 20 – 37°C.
Zero against: water or air.

	Sample A	Sample B
R1	1000 µl	1000 µl
Sample	40 µl	40 µl
Mix and read DO1		
R2	160 µl	160 µl
Mix, wait for 15 minutes and read DO2		

Calculations:

If the expected concentration of L-Ascorbic acid
is between 0 to 300 mg/l use the following calculations:

[Ascorbic acid] mg/l = 625,3 x ΔDO

ΔDO = (DO2-DO1)sample B - (DO2-DO1)sample A