



Most suited bacteria for very acidic wines



As a producer of wine lactic acid bacteria, Lallemand has developed a unique STANDARD $^{\text{TM}}$ production process that allows specific wine bacteria strains under freeze-dried format, to withstand the high acidity of white wines. The STANDARD $^{\text{TM}}$ lactic acid bacteria possess the ability to conduct reliable malolactic fermentation (MLF) in very acidic conditions, with a specific protocol of reactivation and acclimatisation performed prior to inoculation into fermented must or wine.

DESCRIPTION

LALVIN MT01™ is a single strain of *Oenococcus oeni* isolated in a famous area of France for the production of sparkling wine.

LALVIN MT01™ is a freeze-dried wine bacteria produced by Lallemand Oenology with our specific process called "standard process", which allows to produce this efficient starter culture particularly suited to achieve a complete malolactic fermentation in very acidic sparkling wine conditions when used with the appropriate two-step protocol comprising an activation and an acclimatisation steps.



LALVIN MT01™ is also suited for white and rosé wines as well as red wines with low pH and when neutral sensory impact is desired.

PROPERTIES

After appropriate reactivation & acclimatisation steps, LALVIN MT01[™] has the ability to tolerate the following range of wine parameters:

- Alcohol: < 14% v/v
- pH: > pH 2.9
- Total SO₂: < 50 mg/L

For application in sparkling base wine, and to avoid LALVIN MT01 $^{\text{m}}$ inhibition by SO₂, it is recommended that the concentration of measurable total SO₂ in the must/wine prior to inoculation should not exceed 30 mg/L.

- Temperature: > 15 °C (best conditions 18-22 °C)
- Nutrition demand: strongly recommended to add a bacteria nutrient as indicated in the instructions for use
- Low volatile acidity production
- Bacteria cinnamoyl esterase negative: cannot produce precursors for volatile phenol production by *Brettanomyces*
- No production of biogenic amines
- Sensory impact: LALVIN MT01™ is characterised by very low production of diacetyl due to a lack of citrate permease activity and has neutral sensory effect, protecting varietal fruit aromas.

Remark: Due to the numerous inter-relationships existing between wine physico-chemical parameters, the 'cumulative inhibitory effects' of multiple limiting conditions on bacterial growth and activity, such as high temperature with high alcohol, and low pH with high SO₂ must not be ignored.



INSTRUCTIONS FOR OENOLOGICAL USE

Two steps are required to prepare an active, acclimatised starter culture of LALVIN MT01™ for inoculation into must or wine: STEP 1: culture activation step and STEP 2: culture acclimatisation step

Prescribed volume of the activation medium: 0.2% of the total vat volume.

Prescribed volume of the acclimatisation medium: 3% of the total vat volume

Prescribed dose of LALVIN MT01™: 1 sachet of 25 g for 25 hL of total vat volume (final wine volume to be inoculated). The recommended dose of LALVIN MT01™ in these protocols allows to achieve a viable bacterial cell conc. of ≥106 cells/mL, that is required to initiate malolactic fermentation (MLF) and minimise delay to initiate MLF, thus reducing the risk of MLF failure.

◆ STARTER CULTURE PREPARATION WITH MUST TO INOCULATE 25 HL OF WINE

Using the protocol with MUST, the culture activation step (A1) and the preparation of the acclimatization medium (B1) have to be realized at the same time and each of both steps require 3 days, as described below

▶ STEP 1: CULTURE ACTIVATION STEP & PREPARATION OF THE ACCLIMATISATION MEDIUM

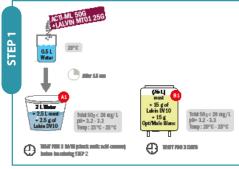
A1 CULTURE ACTIVATION STEP

Prepare culture activation medium as follows in a suitable vessel with airlock or cover

- 2.5 L sterile grape or must or apple juice (low total SO₂: less than 20 mg/L).
- 2 L distilled or chlorine-free water at temperature 25 °C.
- Mix both and adjust to pH 3.2 3.3
- Add 2.5 g active dry yeast (LALVIN DV10™).
- Prepare a homogeneous suspension of bacterial nutrient ACTI-ML™ (50 g) in 500 mL clean, chlorine-free water at 20 °C. Mix in & rehydrate the full amount of one sachet of LALVIN MT01™ (25 g) into the 500 mL ACTI-ML™ suspension. Stir gently and wait for maximum 15 minutes before inoculating the activation medium.

Culture Activation step

- Add this rehydrated bacterial/nutrient suspension into the 4.5 L of culture activation medium.
- Incubate for 72 hours (3 days) at 23-25 °C (CRITICAL STEP), with occasional gentle mixing over the incubation period.
- Culture is ready for transfer into next phase at maximal turbidity and when malic acid is less than 1 g/L (foaminess and a lactic aroma are evident).



B1 During the same time: PREPARATION OF 75 L OF ACCLIMATISATION MEDIUM WITH MUST in a suitable vessel or tank with minimal ullage

- 75 L of must non chaptalised with low total SO₂ (less than 20 mg/L) at temperature at 25 °C.
- Adjust to pH 3.1 3.2
- Start the alcoholic fermentation with 15 g of the active dry yeast LALVIN DV10™ previously rehydrated in a mix must/water (1/2 h at 37 °C).
- Add 15 g of OPTI'MALO BLANC™ previously rehydrated in 200 mL clean water & then mix well into the must.
- Ferment during the 3 days (duration of the activation step) and maintain the temperature of alcoholic fermentation at 20 - 25 °C

STEP 2: ACCLIMATISATION STEP

After 3 days of activation, the activated bacteria culture (5 L) is ready to be inoculated in the acclimatisation medium (75 L) already in fermentation for 3 days (see preparation above).

- Add the whole activation medium (total 5 L) into the 75 L of fermenting wine prepared and mix well. As soon as the alcoholic fermentation is finished, maintain the temperature at 20 °C to achieve the malolactic fermentation.
- Let stand at 20 °C until 2/3rd of the L-malic acid is metabolised, or when the residual L-malic acid approaches 1 q/L. If malic acid analysis on-site is not available to guarantee immediate results, the culture can be assumed to be ready when CO₂ evolution is evident, and a slight lactic aroma is detected (CRITICAL STEP).

Note: if the final wine to be inoculated has difficult MLF conditions (e.g., low pH), it is highly recommended that this step is repeated by sequential doubling of culture volume with wine to allow the bacteria culture to further acclimatise – the total volume will be 160 L



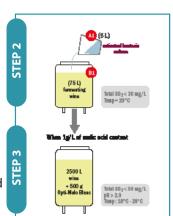
▶ STEP 3: INOCULATION OF ACCLIMATISED CULTURE INTO FINAL WINE

Prepare 2,500 L final wine for inoculation

- Prior to inoculation of the acclimatised culture, ensure that wine physico-chemical properties are appropriate for LALVIN MT01™ (pH > 2.9, total SO₂ < 50 mg/L, alcohol < 14% v/v), and temperature is adjusted to 18-20 °C.
- To ensure an adequate supply of bacterial nutrients and to assist the malolactic culture to survive the final wine environment, we recommend using the bacterial nutrient OPTI'MALO BLANC™ (dose rate 20 g/hL calculated on final wine volume) e.g. for 2,500 L wine, suspend 500 g of OPTI'MALO BLANC™ in 5 L clean water & add to the final wine.

Final inoculation: after the acclimatisation step, the malolactic culture is ready to be transferred into final wine volume with gentle mixing.

- Maintain temperature at 18-20 °C
- Check the degradation of malic acid
- At the end of MLF, rack off and adjust the SO₂.



STARTER CULTURE PREPARATION WITH WINE TO INOCULATE 25 HL OF WINE

Additional precautions are necessary about the risk of oxidation of wines during all the steps. Assure that the vessel or tank are full of wine. In case of mixing, stir gently to avoid excessive oxygenation, and monitor the wine's temperature as indicated below.

▶ STEP 1: CULTURE ACTIVATION STEP

Prepare culture activation medium as follows in a suitable vessel with airlock or cover

- 2.5 L of moderately sulphured wine (no free SO₂ and total SO₂ between 20 and 30 mg/L), with a pH close to 3.2.
- 2 L of hot chlorine-free water to obtain a mixture (wine + water) whose temperature is around 25 $^{\circ}$ C
- Prepare an homogeneous suspension of bacterial nutrient ACTI-ML™ (50 g) in 500 mL clean, chlorine-free water at 20 °C. Mix in & rehydrate the full amount of one sachet of LALVIN MT01™ (25 g) into the 500 mL ACTI-ML™ suspension. Stir gently and wait for maximum 15 minutes before inoculating the activation medium.

Culture Activation step

- Add this rehydrated bacterial/nutrient suspension into the 4.5 L of culture activation medium.
- Incubate for 72 hours (3 days) at 20 °C (CRITICAL STEP), with occasional gentle mixing over the incubation period.
- Maintain temperature at 20 °C until the malic acid content has been totally consumed (<0.2 g/L) then incorporate into the acclimatisation medium prepared for the step 2.

▶ STEP 2: ACCLIMATISATION STEP

Preparation of 75 L of wine for acclimatization step as follows in a suitable vessel with airlock or cover

- Remove 75 L of wine from the vat that has finished its alcoholic fermentation and with a degree of alcohol lower than 13% vol. The pH of the wine should be close to 3.2. If not, de-acidify it using potassium bicarbonate. The amount of total SO₂ should be below 30 mg/L and the content of free SO₂ should be practically zero.
- The temperature of the wine must be between 20 and 22 °C.
- Add 15 g of OPTI'MALO BLANC™ (suspend it in 150 mL clean water & then mix well into the wine)

Then, add at the top of the tank, the activation medium with bacteria starter culture, without stirring.

- Maintain the temperature at minimum 20 °C
- Check the degradation of malic acid, until 2/3rd of the L-malic acid is degraded.
- At this stage, inoculate the final tank of wine according to the protocol indicated below

Note: if the final wine to be inoculated has difficult MLF conditions (e.g., low pH), it is highly recommended that this step is repeated by sequential doubling of culture volume with wine to allow the bacteria culture to further acclimatise – the total volume will be 160 L

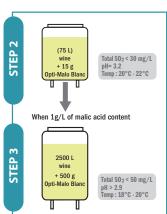
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PACKAGING & STORAGE

- Product in powder form obtained by lyophilisation.
- Available in dosage for 25 to 35 hL (sachet 25 g) and for 500 to 700 hL (sachet 500 g).
- Once opened, wine bacteria sachet must be used immediately.
- This product can be stored for 18 months at 4°C/40°F or 36 months at -18°C/O°F in original sealed packaging.
- Sealed packets can be delivered and stored for 3 weeks at ambient temperature (<25 °C/77 °F) without significant loss of viability.

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The information in this document is correct to the best of our knowledge. However, this data sheet should not be considered to be an express guarantee, nor does it have implications as to the sales condition of this product. January 2022.















