USER GUIDE

Encapsulated Dry Yeast
for Sparkling Wine
1. Proelif description

Proelif is a dehydrated product in the form of 2mm diameter beads, composed of *Saccharomyces cerevisiae* yeasts enclosed in an alginate capsule. The capsule’s external layer of alginate prevents the release of the yeast cells into the wine (picture 1 and 2).

Picture 1 – Photo of a bead’s interior, taken with an electronic Microscope (courtesy Univ. Minho,Portugal).

Picture 2 – Drawing of an encapsulated yeast

2. Why use Proelif?

No riddling needed.

In sparkling wine production using the *Méthode Traditionnelle*, a second alcoholic fermentation occurs in the bottle after the inoculation of a yeast suspension. At the end of alcoholic fermentation, the sediment and the yeast’s elimination require long and expensive procedures – Riddling.

The yeast encapsulation procedure allows the yeast (after the beads introduction into the bottle together with the base wine) to perform its fermentative activity inside the capsule via the entrance of substrates and the release of metabolites. This occurs without releasing the yeast cells into the wine (Picture 2). Proelif beads drop quickly when inverting the bottle, making them easier to be removed by *disgorging*, without requiring riddling.
3. Base Wine Profile

As in the sparkling wine production with free yeasts, the base wine must have certain characteristics for the positive alcoholic fermentation performance in the bottle.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular SO₂</td>
<td>≤ 0,7 ppm</td>
</tr>
<tr>
<td>pH</td>
<td>≥ 3,0</td>
</tr>
<tr>
<td>Alcohol</td>
<td>≤ 11,5% (v/v)</td>
</tr>
<tr>
<td>Yeast available nitrogen (YAN*)</td>
<td>≥ 100mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>&lt; 80mg/L</td>
</tr>
<tr>
<td>No antimicrobial agents (velcorin, sorbate) added.</td>
<td></td>
</tr>
<tr>
<td>Fermentation temperature</td>
<td>&gt; 12ºC</td>
</tr>
<tr>
<td>it is not advisable to exceed 24ºC</td>
<td></td>
</tr>
<tr>
<td>Protein stability</td>
<td></td>
</tr>
<tr>
<td>Tartaric stability**:</td>
<td></td>
</tr>
<tr>
<td>Saturation temperature:</td>
<td></td>
</tr>
<tr>
<td>White wine</td>
<td>&lt; 10ºC</td>
</tr>
<tr>
<td>Red and rose wine</td>
<td>&lt; 16ºC</td>
</tr>
</tbody>
</table>

* 100 mg/L of ammonium phosphate is equivalent to 21 mg/L of YAN

** Tartaric stabilization by electrodialysis and / or cationic resins may result in a significant reduction of pH. After using these techniques, it is important to verify that pH ≥ 3.0.

Alcohol, pH, SO₂ and temperature act in synergy. If the values of these parameters are to close to the limit, the fermentative kinetics will be slower.

It is important to verify the sugar content of the base wine, in order to calculate the volume of *liqueur de tirage* to be added on the eve or on the *tirage* day.

**Tartaric stability**

Our trials show that the beads tend to agglomerate in wines that do not have a proper tartaric stability, which does not allow an efficient disgorging.

Therefore, we recommend the following saturation temperature values (Stabisat equipment) after tartaric stability procedures:

- White wines: Saturation temperature <10ºC;
- Red and rosé wines: Saturation temperature <16 ºC

All tartaric stability methods are compatible with the use of Proelif however there are some safeguards to take into account:

- Electrodialysis and/or cationic resins stabilisation can lead to an important reduction in pH. After using these techniques it’s important to check that the final pH of the wine is pH ≥ 3,0.

**Protein stability**

The base wine must be protein stabilized in order to avoid insolubilizing the unstable proteins during the second fermentation. Protein stability prevents turbidity/deposit in the bottle. The most effective way to eliminate unstable proteins is through the bentonite application. This treatment is most effective and less damaging to the wine quality if applied during the first fermentation.
Fermentation test

It’s recommended to perform a bottle fermentation trial at laboratory scale to evaluate the fermentation kinetics of the base wine. This test must simulate the procedure used in the tirage. Follow the pressure evolution in the bottle. At 14°C the pressure it’s expected to increase by 0.1 bar/day from the 5th day and up to 4 bar.

4. Hygiene Equipment Protocol

As in the sparkling wine production with free yeasts, the hygiene procedures of the equipment’s are very important for the sparkling wine quality.

When using Proelíf strict attention to hygiene of all material and equipment used during tirage (appendix I) is crucial. This procedure is very important to prevent the growth of microorganisms that may cause bottle cloudiness. If possible, proceed settling with a hygiene control using classic microbiology or quick methods (i.e. bioluminescence). The control of chemical product residues used in cleaning and disinfection is also important since even in small quantities of sanitizing agents have a microbicide action which may interfere with Proelíf. After using the production line for the tirage with free yeasts it is recommended a stricter cleaning and disinfection.

The adoption of the following cleaning and disinfection protocols it’s mandatory to ensure the success of the application. The choice between one or another depends on the availability of cleaning/disinfection products and steam generator. The protocols are appropriate to the following circuit:
Filter -> Post-filter pipes or flexible hoses -> Filler

For cleaning and disinfection, we recommend to use:
- A detergent made of caustic soda (NaOH) or caustic potash (KOH)
- A disinfectant made of peracetic acid

Always use the concentrations recommended by the manufacturer of the hygiene products. Before starting the tirage we always recommend to check the filler seals and joints. If they show signs of abrasion it’s convenient to replace them.
CHEMICAL PROTOCOL

Before tirage:
1. Rinse the circuit with water;
2. Run a detergent solution at 60°C during 20 min.
3. Rinse with water and make sure there are not any residues of the cleaning agent.
4. Run a disinfection solution at room temperature during 20 min.
5. Rinse with water at room temperature and make sure there are not any residues of the disinfectant agent.
6. When available apply cleaning foam on the filler and on the filling spouts. If foam is not available pulverise the filling spouts with alcohol.
7. Hand clean with alcohol the doser and the bowl of the dosing unit.

End of tirage day: 
1. Rinse the circuit with water.
2. Run a detergent solution at 60°C during 20 minutes.
3. Rinse with water at room temperature and make sure there are not any residues of the cleaning agent.
4. Run a disinfectant solution at room temperature during 10 minutes. Leave the circuit (filter + tubes + filler) on charge until the next morning.
5. Hand clean with alcohol the doser and the bowl of the dosing unit.

Beginning of the next tirage day: 
1. Rinse the circuit with water at room temperature and make sure there are not any residues of the disinfectant agent.

PHYSICOCHEMICAL PROTOCOL

Before tirage:
1. Rinse the circuit with water;
2. Run a detergent solution at 60°C during 20 minutes.
3. Rinse with water at room temperature and make sure there are not any residues of the cleaning agent.
4. When available apply cleaning foam on the filler and on the filling spouts. If foam is not available pulverise the filling spouts with alcohol.
5. Inject steam in the circuit. Once the steam starts to come out of the filling spouts leave it flowing during 20 minutes.
6. In order to cool down the material, rinse the circuit with water or with wine at room temperature
7. Hand clean with alcohol the doser and the bowl of the dosing unit.

End of tirage day: 
1. Rinse the circuit with water;
2. Run a detergent solution at 60°C during 20 minutes.
3. Rinse with water at room temperature and make sure there are not any residues of the cleaning agent.
4. Hand clean with alcohol the doser and the bowl of the dosing unit.

Beginning of the next tirage day: 
1. Inject steam in the circuit. Once he steam starts to come out of the filling spouts leave it flowing during 20 minutes.
2. In order to cool down the material, rinse the circuit with water or wine at room temperature
3. Hand clean with alcohol the doser and the bowl of the dosing unit.
5. Tirage Day

5.1. Addition of “liqueur de tirage”

It must be added to the wine at the beginning of the tirage day or in the day before. As the riddling step is no longer required, it is not necessary to add riddling adjuvants. Thiamine, DAP as well as tannins or proteins (to enhance volume and mouthfeel) should be added before the final filtration.

5.2. Filtration

The wine must be filtered using a 0.45 or 0.65µm absolute pore membrane filter. It is essential that the filtration procedure is followed properly. Test the membrane integrity before and after each bottling cycle and respect the differential pressure recommended by the cartridge supplier. Filtration must be performed on the bottling day and directly to the filler in order to avoid any post filtration contamination. In white and rosé wines without malolactic fermentation use 0.45 µm membrane.

5.3. Dosing Equipment

The beads are introduced directly into the bottle during the bottling process. The Proelif dosing equipment can be manual or automatic. The equipment can be placed before or after the bottle filler (Appendix 1) depending on space available to fit it. If the dosing unit is placed after the filler it is important to monitor the bottleneck after the dosage to be sure that the beads do not stick on it.

The beads dosage must be 1.2 to 1.5 g/bottle (depending on the wine analyses profile), meaning 4 to 6 millions of viable cells/mL of wine. To ensure a reproducible dosing into the bottles, the Proelif must be at room temperature. The quantity of Proelif to be used in the next day has to be taken out of cold storage (4±2ºC) in the evening. This procedure prevents water condensation on the product and guarantees a good flow of Proelif, avoiding irregular dosages. At the beginning of the working day we advise to check if the dosage is in the desired value. For that purpose a dozen of empty bottles and run it through the dosing unit. Weigh the quantity of Proelif in each of those bottles.

5.4. Base wine temperature and bottles temperature

In the tirage day the base wine and the bottles must be between 13 and 20ºC, preferably around 16ºC.

6. Bottle storage

Store the bottles in a location free from temperature fluctuations and air currents.

- Fermentation temperature: 12 < $T$ (°C) < 24

7. Application check list

To be sure that all requirements prior to bottling are fully complied, use the Check List in appendix 2.
8. Fermentative kinetics control

We suggest a regular analysis of the following fermentation parameters:

1. Storage temperature;
2. Pressure;
3. Sugar;
4. Bottle visual monitoring

We recommend keeping control bottles, meaning bottles with and without ProElif® (Witnesses), which must be stored at the same temperature as the other bottles. This procedure will be very useful to identify the origin of contamination, in case of cloudiness in bottles.
* If there is a lack of space, the dosing equipment can be placed after the bottle filler. However, in that case, it is important to monitor the bottleneck after the dosage to be sure that the beads do not stick on it.
APPENDIX II

Proelif Application Check List

1. **Chemical analysis of the base wine**

<table>
<thead>
<tr>
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<th>Result</th>
<th>Limit</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Alcohol (%v/v)</td>
<td></td>
<td>≤ 11.5</td>
</tr>
<tr>
<td>Yeast Available Nitrogen – YAN (mg/L)</td>
<td></td>
<td>≥ 100</td>
</tr>
<tr>
<td>Calcium (mg/L)</td>
<td></td>
<td>&lt; 80</td>
</tr>
<tr>
<td>Protein stability</td>
<td></td>
<td>Stable</td>
</tr>
<tr>
<td>Tartaric stability</td>
<td></td>
<td>Stable</td>
</tr>
<tr>
<td>- Saturation temperature in white wines (°C)</td>
<td></td>
<td>&lt; 10</td>
</tr>
<tr>
<td>- Saturation temperature in red and rosé wine (°C)</td>
<td></td>
<td>&lt; 16</td>
</tr>
</tbody>
</table>

2. **Addition of nutrients and liqueur de tirage to the wine:**

DAP (if YAN<100mg/L) ________ g/hL

Thiamine 0.3 mg/L

Nutrients and liqueur de tirage added to the wine before filtration and in eve or tirage day

Wine free of antimicrobial agents (velcorin, sorbates...)

3. **Filtration**

Final filtration filter with 0.45 or 0.65 microns absolute pore size

Filter integrity test

Filtration processed in line and wine sent directly to the filler

Filter differential pressure doesn’t exceed manufacturer’s recommendation

4. **Equipment cleaning and disinfection (filters + pipes + filler)**

<table>
<thead>
<tr>
<th>Chemical protocol:</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before starting tirage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beginning of the day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of the day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physicochemical protocol:</th>
<th>Yes</th>
<th>No</th>
</tr>
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<tbody>
<tr>
<td>Before starting tirage</td>
<td></td>
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<td>End of the day</td>
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<td></td>
</tr>
</tbody>
</table>

**Hygiene control results:**

5. **ProElif® dosage ≥ 1.2 g/bottle**

6. **Base wine temperature and bottles temperature > 13°C**

7. **Fermentation temperature ≥ 12°C**

8. **Control bottles (with and without Proelif)**

9. **Notes**