





# When to use this procedure

This protocol outlines the steps required for the preparation of a 'rescue' culture of yeast for a slow or stuck fermentation. It is based on the collective experience of winemakers, AWRI researchers and industry suppliers of yeast.

This protocol is recommended for ferments which exceed 12% v/v alcohol and more than about 5-10 g/L residual sugar, and especially when the ferment has not responded well to standard interventions, such as aeration, nutrient additions, addition of yeast hulls or addition of additional yeast inocula. The aim of this scale-up procedure is to acclimatise the yeast to the relatively hostile conditions of the ferment.

For ferments containing lower concentrations of alcohol and sugar, it is recommended to attempt rescue using yeast lees from a recently completed successful ferment or with an active yeast culture freshly prepared by propagating yeast in grape juice by a standard procedure, rather than following this procedure, which requires significant time and resources.





# Preliminary steps to render the problem wine most suitable for rescue

- Check viability of the yeast in the stuck ferment (See Iland et al. 2004). A value of less than 60-70% confirms a toxic environment.
- If malolactic fermentation (MLF) has commenced, it is advisable to wait until MLF has completed. Then add sufficient sulfite to achieve a free concentration of 30 mg/L (at pH 3.5) and leave for at least 24 hours. If necessary, treat with bentonite and rack.
- Blanket the wine with inert gas to prevent oxidation.
- If possible, rack the wine to remove sedimented yeast, which can interfere with rescue.
- Check volatile acidity (VA). If VA> 1.0 g/L, then this also creates a toxic environment and VA reduction should be considered prior to rescue.
- Check alcohol concentration. If alcohol exceeds 15 % v/v, a maximum recommended hulls addition should be considered. (Warning ensure that hulls are fresh and are not stale/rancid/tainted; confirm by sensory evaluation of double amount steeped in clean wine for several hours.)
- Consider an addition of proprietary yeast hulls to aid re-fermentation by a combination of adding micronutrients and adsorbing toxic substances.
- Measure YAN and adjust to 20 mg/L YAN (100 mg/L DAP provides 20 mg/L YAN). Adding recommended amount of proprietary fermentation nutrient can be beneficial, taking into account total YAN.
- Consider use of a fructophilic rescue yeast, given that most stuck ferments have higher residual fructose than glucose.

# **Rescue procedure**

The protocol below is appropriate for the preparation of yeast to re-inoculate a 1000 L problem ferment and may be scaled up for larger volumes.

- 1. Warm the bulk ferment to 18-22°C.
- 2. Prepare the reactivation medium. This can be based on grape juice, if available, or a mixture of grape juice concentrate, water and nutrients. In either case, it should be adjusted to 20°C before use. The two 'recipes' are given below:





Grape juice reactivation medium	Concentrate/water reactivation medium
10 L grape juice (free of SO2)	15 L clean water (free of chlorine)
5 L clean water (free of chlorine)	3 kg grape juice concentrate (GJC is measured by weight, not volume; this amount is equivalent to ~2 kg sucrose since GJC is
15 g DAP	approximately 67% sugar)
Proprietary fermentation nutrients at 10x recommended rate (sterols)	15 g DAP
	Proprietary fermentation nutrients at 10x recommended rate (sterols)

- 3. Rehydrate the yeast. Use double the recommended normal yeast inoculum. For 1000 L, rehydrate 500 g active dried yeast by suspending in 5 L clean water (free of inhibitory substances such as chlorine) at 38-40°C for 15 min. Do not stir at this stage, other than to ensure that the yeast is completely wetted when initially added. The most successful culture according to feedback from winemakers is that marketed as *PDM*, or *Prise de mousse*. Suppliers often recommend fructophilic yeasts such as *Lalvin Uvaferm 43*, *Maurivin Elegance*, or *Oenoferm Freddo*. Consult yeast suppliers for their recommended strain and reactivation procedure for the particular ferment conditions.
- 4. After the rehydration period, add the reactivation medium (15 L) gradually in several small volumes, to cool the culture (see table below), with each addition separated by five minutes. Mix carefully after each addition. Leave the reactivated culture to stand until approximately 50% of the residual sugar has been consumed (monitor by hydrometry). This is likely to take approximately four hours (see table below).
- 5. At this point, add 20 L of the problem ferment, and aerate at a rate of 0.01-0.1 volumes filtered air/volume liquid/min (i.e. if the volume is 40 L, the flow rate should be in the range 0.4-4 L air/min.). If possible, use agitation with a stirrer bar/overhead mixer to keep the culture in suspension and facilitate oxygen transfer. An air compressor, microoxygenation equipment or a cylinder of instrument-grade, filtered, compressed air should be used for the aeration step. Oxygen is an essential nutrient which helps the yeast cell membranes keep their integrity at higher alcohol concentrations. If aeration is not possible, then a nutrient mix containing sterols should be used in place of air/oxygen.
- 6. Monitor the concentration of residual sugar hourly. When approximately 50% has been fermented, double the volume again with the problem ferment. Continue aeration.
- 7. This step should be repeated at least twice, to ensure that the alcohol concentration is increased gradually, to allow the yeast to acclimatise.
- 8. Add the yeast culture to the tank and mix. Approximately 20 mg DAP/L may be added to the bulk ferment when the culture is added. Maintain the ferment at 18–22°C, and agitate or pump over at least once a day, to prevent the yeast from settling out. Aeration of the ferment may be





- risky, as the yeast may not be sufficiently active to prevent oxidation. When fermentation is evident, limited aeration can be beneficial (equivalent to  $5 \text{ mg/L } O_2$ )
- 9. Check the residual sugar concentration at least daily. At this stage a more precise measure is required: use *Clinitest* or another method. If the rate of fermentation is very low (less than 1 g/L/day), please contact the AWRI for more assistance.

#### The following table summarises the size and timing of the additions made:

Stage	Cumulative time (min)	Temp. (°C)	Volume added (L)	Cumulative volume (L)	Comment
Rehydration	0	40	5	5	Do not stir
Cooling	15	~35	2	7	Add rehydration medium and mix
Cooling	20	~30	3	10	As above
Cooling	25	~25	5	15	As above
Cooling	30	~20	5	20	As above
Acclimatisation	~240	~20	20	40	Add problem ferment, and aerate continuously
Acclimatisation	~600	~20	40	80	As above
Acclimatisation	(when ~50% residual sugar remains)	~20	80	160	May continue with more stages as above

Note that the duration of this process is variable, and the time elapsed should not be used as a guide to the timing of the addition of more ferment. The rate of sugar usage is far more important. The total time, from rehydration of the yeast to inoculation of the bulk ferment, may be 12–72 h.

The large yeast population present means that the sugar present is likely to be rapidly metabolised, and care must be taken to ensure that the yeast do not ferment all of the residual sugar at any stage. If the culture 'runs dry' a rapid loss of yeast activity can be expected and a new batch should





be prepared. Also ensure that the YAN at each culture stage does not fall below 20 mg/L YAN, since rapid loss of yeast activity can be expected and a new batch should be prepared.

In particularly difficult cases, winemakers have had success by adding the bulk ferment to the final stage of the rescue culture in successive increments, rather than adding the rescue culture to the tank. Under these circumstances, it is necessary to transfer a volume of the warm ferment to the tank containing the culture on a daily or twice-daily basis, ensuring that the residual sugar concentration is determined before and after each addition, so that the rate of fermentation can be monitored.

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## References and further reading

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