

## Cryoconservation of LEXSY

Glycerol stocks may be prepared from standard cultivation in **LEXSY BHI medium** for strain maintenance after 3 days (1:20 dilution) or after 4 days (1:50 dilution) TC flasks upright at OD 1.4-2, ca.  $6-8 \times 10^7$  cells/ml (ref. to **capt. 3.2.**). Alternatively, stocks can be prepared from 1:10 inoculated cultures after 24 h if TC flasks are incubated flat. Avoid to prepare stocks from cultures not dense enough or from late stationary phase cultures. Prior to conservation, check vitality of cells by microscopy. Cells should be motile and elongated but not of “needle-like” appearance. Do not use other media than **LEXSY BHI** for cryoconservation.

### Glycerol stocks preparation using a cryocontainer

Using this method, refrigeration proceeds continuously with  $-1^\circ\text{C}/\text{min}$

- Add 1.2 ml autoclaved Glycerol (80% by weight) to a sterile 15 ml Falcon tube
- Withdraw 3.6 ml of culture OD 1.4-2 (ca.  $6-8 \times 10^7$  cells/ml)
- Mix with glycerol and distribute 3 x 1.6 ml each to sterile cryovials
- keep 10 min at room temperature
- transfer to a Cryocontainer at  $4^\circ\text{C}$  containing Isopropanol
- keep 10 min at  $4^\circ\text{C}$
- transfer to  $-80^\circ\text{C}$  over night
- distribute to storage box for long term storage

### Glycerol stocks preparation by stepwise cool-down protocol

Using this method, refrigeration proceeds in steps of  $0^\circ\text{C}/-20^\circ\text{C}/-80^\circ\text{C}$

- Add 1.2 ml autoclaved Glycerol (80% by weight) to a sterile 15 ml Falcon tube
- Withdraw 3.6 ml of culture OD 1.4-2 (ca.  $6-8 \times 10^7$  cells/ml)
- Mix with glycerol and distribute 3 x 1.6 ml each to sterile cryovials
- keep 10 min at room temperature
- keep 1 h on wet ice
- keep o/n at  $-20^\circ\text{C}$
- transfer to  $-80^\circ\text{C}$  for long term storage

Both protocols are tolerated well by *L. tarentolae*. However, to avoid loss of strains it is recommended to check the reactivation of one sample of glycerol stocks prepared prior to stopping respective suspension cultivation.

## Glycerol stocks reactivation

- Thaw frozen glycerol stock on ice (ca. 20 min)
- Inoculate the **entire content** of the vial into 10 ml of **LEXSY BHI** medium with appropriate antibiotic(s). *Motile cells can be observed immediately after inoculation by microscopy*
- Incubate as static suspension culture in ventilated TC flask (flat) dark at  $26^\circ\text{C}$  until culture gets turbid (OD 1.4-2; ca.  $6-8 \times 10^7$  cells/ml). This usually takes 2 days; wait longer if cells recover more slowly and follow status by microscopy

Dilute dense culture 1:10 into fresh **LEXSY BHI** and incubate for 3 days. Do not dilute culture of low density. For strain maintenance dilute into fresh **LEXSY BHI** on Monday and Friday each week (see capt. 3.2.).