**Maintenance & Culture of *L. tarentolae* T7-TR strain (Jena Biosc)**

**Media :**

* LEXSY-BHI (ML-412S). Stable 12 months RT. 37g/L. Dissolve by agitation and then Autoclave precisely 121°C 15’. From this prepare fresh media (small tubes at the time, since media is viable 2 weeks after Hemin addition) and keep in aluminum foil at 4°C.
* HEMIN (0.25% porcine in 50% Triethanolamine ; ML-108S). At 4°C in the dark.
* Pen-Strep 200X (ML-105S). -20°C in Aliquots
* LEXSY NTC 1000X (AB-101S)
* LEXSY-Hygro 1000X (AB-104S)

**Culturing:**

- All culturing is carried out at 26°C in the dark under aerated conditions (no CO2 incubator required) in upright ventilated flasks in minimum 10ml.

- Dilute 1:10 for 2 das confluency and 1:20 for weekend passage.

NOTES :

- Avoid repeated successive dilution of cultures of lower cell densities as this may reduce growth

- Do not use agitation for strain maintenance

- To centrifuge cells do it at 2000g x 3’ RT.

**Storage:**

- From a 1:10 well grown cultures, motile and drop-like cells (NOT as needle-like appearance).

1. Add 1.2 ml autoclaved Glycerol (80% by weight per volume) to a sterile 15 ml Falcon tube
2. Withdraw 3.6 ml of culture OD 1.4-2 (ca. 6-8 x107 cells/ml)
3. Mix with glycerol and distribute 3 x 1.6 ml each to sterile cryovials
4. Keep 10 min at room temperature
5. Transfer to a cryo container at 4°C containing fresh isopropanol. Keep 10 min at 4°C
6. Transfer to –80°C over night
7. Distribute to storage box for long term storage

- To re-start cultures from -80°C

1. Thaw frozen glycerol stock on ice (ca. 20 min)
2. 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°C until culture get turbid (OD 1.4-2; ca. 6-8 x107 cells/ml). This usually takes 2 days; wait longer if cells recover more slowly and follow status by microscopy