

Standard protocol for transfection of *L. tarentolae* in BHI

- On Friday inoculate *L. tarentolae* pre-culture 1:20 in 10 ml **LEXSY BHI*** medium (**Cat.-No. ML-411**) and incubate in tissue culture (TC) flask upright @ 26°C dark until Monday. Add **LEXSY NTC** and **LEXSY Hygro** for cultivation of **T7-TR** strain for inducible expression throughout the procedure
- On Monday dilute pre-culture 1:10 in 10 ml **LEXSY BHI*** medium and incubate in TC flask flat @ 26°C o/n
- On Tuesday check cell density of the culture until ca. 6×10^7 cells/ml are reached (OD 1.4)** and ensure by microscopy that the cells are vital and of droplike shape
- Spin cells 3 min, 2000g at room temperature and remove ½ volume of supernatant
- Resuspend pellet in remaining medium to get **10^8 cells/ml** and put on wet ice for 10 min
- Have ready on wet ice in parallel tubes with 0.1 - 5 µg transforming DNA in max. 50 µl water or Tris buffer pH 8.0 and electroporation cuvettes d=2 mm***
- Add 350 µl pre-chilled cells to the tube with DNA and transfer to the electroporation cuvette on wet ice
- Electroporate @ **450V, 450µF** and monitor pulse time (ca. 5-6 msec)****
- Put cuvette back on ice for exactly 10 min
- Transfer electroporated cells with capillary to 10 ml **LEXSY BHI*** in a ventilated TC flask
- Incubate o/n @ 26°C as static suspension culture (ca. 20h, OD 0.3-0.4)
- Proceed with clonal or polyclonal selection (**capt. 5.1.** and **5.2.** of LEXSY Expression Kit manuals)

* contains Hemin,

** if the cell density differs from this value, concentrate cells in the next step in such a way to get **10^8 cells/ml**. For transfection cultures between OD 1.0-1.8 can be used. Avoid to transfect cells if they are long and thin

*** use electroporation cuvettes with long electrodes. The entire volume of 0.4 ml must be between the electrodes. Do not use electroporation cuvettes with short electrodes leaving most of the 0.4 ml outside of the linear electric field

**** using BioRad GENEPULSER II with PULSE CONTROLLER II and CAPACITANCE EXTENDER PLUS or GENE PULSER Xcell with PC and CE Modules. The resistance of the sample is 20 Ohms. With GENE PULSER Xcell you may alternatively use the **Time constant protocol** with the settings **450 V and 3.5 ms** (Appendix 8.4. of LEXSY Expression Kit manuals)

Please, refer to **capt. 4.4.** of LEXSY Expression Kit manuals for more details. Appendix 8.4. of the manuals describes also an alternative **High voltage protocol** for transfection of LEXSY.