How to grow a *Leishmania* culture

- *L. tarentolae* needs **aerobic** conditions. The strains can be maintained as continuous suspension culture with regular dilutions (see chapter 3.2 of LEXSY manuals). All cultivations are performed **at 26°C**. Higher temperatures lower the growth-rates and vitality significantly and *L. tarentolae* will not survive at 37°C

- All growth media should be supplemented with **Hemin** (Cat. No ML-108) which is essential for *Leishmania*. Hemin is light-sensitive, so *Leishmania* must be cultivated in the dark. After completion with Hemin the medium must be stored in the dark at 4°C. For optimal growth and vitality the completed medium should be used within 2 weeks. However, if this shelf live is exceeded, it is possible to re-add Hemin (and PenStrep) and to use this medium for 2 more weeks

- For **maintaining** LEXSY strains for transfection and analysis it is convenient to grow static suspension cultures in 10 ml **LEXSY BHI medium** (Cat. No. ML-411, 412) in ventilated tissue culture (TC) flasks. Don´t use agitated cultures for strain maintenance since cells will age much faster. It is not necessary or growth-promoting, to add serum to the BHI medium

- Best results are obtained with inoculations during early stationary phase. Avoid repeated successive dilution of cultures of low cell densities as this may drop growth. However, occasional higher dilutions of stationary cells at e.g. 1:100 will not adversely affect subsequent growth. It is convenient to dilute 10 ml cultures 1:50 on Monday and 1:20 on Friday and to incubate TC flask upright, lowering aeration for longer intervals between passages. Don't cultivate *Leishmania* much longer than for 7 days in the same medium without dilution. For cultivation for transfection see chapter 4.4, for cultivation for protein expression see chapter 5.4 of the LEXSY manuals

- Always control appearance and motility of the cells by microscopy. Cells of mid-growth phase cultures are of drop-like shape, approx. 15x5 µm in size with one flagellum at the flat end, and motile. **These cells are most efficient for transfection and plating on solid media**. Mid-growth phase cultures always contain subpopulations of non- or less motile cells and of cells of different shape. Don't hesitate to transfect, plate or preserve a culture with drop-like cells containing such subpopulations. Cells of older cultures get longer and thinner (needle-like shape) and remain motile. Enhanced motility may result from nutrient deprivation or other limitations and must not necessarily be a sign of mid growth culture stage. Also, bacterial, fungal or other contaminations may be identified by microscopy

- Keep patient, esp. if you are used to working with bacteria. *Leishmania* cells are protozoans with regular doubling times of 7 h in static suspension cultures and 4-5 h in agitated cultures. They need their time to grow or to adapt to new conditions

- If you - despite following these instructions - encounter growth problems with the host strain, sediment cells 3 min at 2000g, resuspend pellet carefully in fresh growth medium and continue incubation in ventilated TC flasks. This approach was very helpful in rescuing cultures esp. after transfection

- Don't centrifuge *Leishmania* cultures at high speed >10.000g and don't resuspend cell pellets by rigorous vortexing. The cells are sensitive to these procedures and may lyse. Centrifugation at 2000-3000g is sufficient for sedimentation and makes gentle and quick resuspension of cell pellets easier. If required, prolong centrifugation time rather than using higher speed

- If you cultivate LEXSY strains in bioreactors be careful with stirring. We found it sufficient to aerate the culture in a 10 L fermentation without stirring for obtaining high cell densities up to 10^9 cells/ml. If you intend to use a stirrer, avoid high sharing forces

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