Data sheet

Leishmania tarentolae T7-TR strain

expressing bacteriophage T7 RNA polymerase and TET repressor, use for inducible LEXSY expression vectors EGE-220 and EGE-221

Cat.-No.	Size
LT-110	3 vials

Description:

The *Leishmania tarentolae* T7-TR strain constitutively expressing bacteriophage T7 RNA polymerase and TET repressor is used as host strain for the inducible LEXSY expression vectors. Please, refer to corporate website for vector details.

EGE-220 EGE-221

Shipping conditions:

The strain is shipped on dry ice.

Product description:

Product LT-110 contains:

- 3 vials with 1.6 ml each of frozen glycerol stocks of *Leishmania tarentolae* laboratory T7-TR strain
  These stocks can be stored at -80°C for at least 1 year.
  For reactivation see below.

Organism: *Leishmania tarentolae* T7-TR strain
constitutively expressing bacteriophage T7 RNA polymerase and TET repressor

Biosafety level: 1; Non–pathogenic for humans

Source: *Tarentolae annularis*

*NOTE: Additional **LEXSY supplements** and components not included in this product are separately available at Jena Bioscience and indicated with:* Cat.No.

Glycerol stocks reactivation:

To reactivate the glycerol stocks provided, thaw on ice and inoculate the **entire content** of one vial into 10 ml **LEXSY Broth BHI** with **Hemin** in a ventilated cell culture flask. The antibiotics **LEXSY NTC** (Nourseothricin) and **LEXSY Hygro** (Hygromycin) are added at 100 µg/ml concentrations to maintain the T7 RNA polymerase and TET repressor genes stably integrated into the host genome. Motile cells can be observed immediately after inoculation by microscope. Incubate at 26°C and dilute 1:5 to 1:10 into fresh medium with antibiotics during mid growth phase (OD ca. 2, approx 2 days post inoculation).

LEXSY cultivation:

L. tarentolae T7-TR can be cultivated in the dark at 26°C in complex media (**LEXSY Broth BHI**) or chemically defined media (**Synthetic LEXSY Broth**), both supplemented with **Hemin** which is essential for *Leishmania* and antibiotics **LEXSY NTC** and **LEXSY Hygro** for maintaining the genes for T7 RNA polymerase and TET repressor stably integrated into the host genome. There is no need to add sera to complex media. Addition of fetal calf serum will not enhance growth of *L. tarentolae* in complex media. To prevent bacterial infections, Penicillin and Streptomycin (**Pen-Strep**) may be added.

ML-411
ML-103
ML-105
ML-108

For alternative complex growth media ref. to the corporate website.

ML-421
ML-431

If you eventually should encounter growth problems with the host strain during or after reactivation from glycerol stocks, sediment cells 5 min at 2000g, resuspend pellet carefully in fresh growth medium and continue incubation in ventilated cell culture flasks.

Cultivation conditions:

L. tarentolae needs aerobic conditions for development.
The strain can be maintained as continuous suspension culture with regular dilutions at 1:10 to 1:50 rates. Best results are obtained by resuspension during mid-late growth phase (OD 2-3). Avoid to inoculate from late stationary phase.

Cultivation can be performed in

- ventilated **cell culture flasks** for suspension cultures, culture V = 10 to 200 ml — EGE-301
- Erlenmeyer flasks, agitated in an incubator at approx. 170 rpm; culture volume of 50 ml to 1 liter — EGE-302
- standard bioreactors up to 100 liter. — EGE-303

For further instructions for handling suspension cultures of *Leishmania tarentolae* please refer to the Appendix **"How to grow a *Leishmania* culture"**, the User's Guide for **LEXSY Gene Expression Starter Kits EGE-120 and EGE-121** and **LEXSY Grower's FAQs** you may download from the corporate website

Storage of *Leishmania tarentolae*

Leishmania tarentolae cells may be stored at -80°C in 20% glycerol for at least one year. We recovered viable cells under these conditions after 4 years.

Glycerol stocks preparation:

- Withdraw 1.2 ml of culture (mid to late growth phase OD_{600nm} in the range of approx. 1.5 - 2.5)
- add 0.4 ml autoclaved Glycerol (80%)
- mix well, keep 10 min at room temperature
- keep 1 h on ice
- keep o/n at –20°C
- transfer to –80°C

Appendix

How to grow a *Leishmania* culture

(1) For **maintaining** LEXSY strains for transfection and analysis static *Leishmania* suspension cultures should be grown in 10 ml **LEXSY Broth BHI** with **Hemin** in ventilated cell culture flasks. Don´t use agitated cultures for strain maintenance as they will age much faster.

(2) For the vitality of *Leishmania* cultures it is important to support continuous growth by regularly transferring the strain at OD_{600nm} in the range 2 - 3 (mid-late growth phase 1 – 2 $x10^8$ cells/ml) into fresh medium at 1:10 to 1:50 dilutions. Use 1:10 dilutions during the week and 1:50 dilutions for maintaining the culture over the weekend, if appropriate. A 1:10 inoculated static suspension culture in ventilated cell culture flasks is ready for the next dilution usually after 3 days, a 1:50 inoculated culture after 5 days. To slow down growth rates you may position the TC flasks upright standing after initial growth for 3 days (lowering aeration), if longer intervals between passages are required. Don't cultivate *Leishmania* longer than 7 days in the same medium when using 1:50 dilutions.

(3) The growth medium should be always fresh prepared. To reach optimal growth and vitality the completed medium should be used within 2 weeks. However, the separate stock solutions of the medium components can be stored for a longer time (Hemin stock up to 1 year at 4°C, Nourseothricin stock up to 6 month at 4°C). If selecting, add the antibiotic immediately before inoculation. Don't use media, where the antibiotic was added a week ago.

(4) **Hemin** in the growth medium is essential and light sensitive → *Leishmania* must be cultivated in the dark and the completed medium with Hemin must be stored in the dark (4°C).

(5) *Leishmania* cells like to be in contact with each other → never inoculate *Leishmania* too rare! Too high dilution may kill the culture, esp. if the starting culture was not yet dense enough (below OD 1; 10^7 cells/ml) or had already passed maximal density OD 3 - 4 and is in the stationary phase or even with decreasing OD. It is a good idea, to use in parallel several dilutions (e.g. 1:10, 1:20 and 1:50) for maintaining the strains, esp. if you prepare cultures for transfection (ref. to the description in the LEXSY expression kit manuals).

(6) **Inspect the static suspension cultures regularly and carefully resuspend the sedimented cells on the bottom of TC flasks by moving them gently**. The cultures should appear cloudy.

(7) For back-ups and in case of longer absence from the lab prepare glycerol stocks from the recipient strain and from your cell lines as described in the LEXSY kit manuals. You may store mid growth phase cultures also up to 2 weeks at 4°C, but there is no guaranty they recover any time and glycerol stocks are always advised.

(8) Glycerol stocks are best prepared from mid growth phase cultures @ $5x10^7$ – $1x10^8$ cells/ml; OD_{600nm} 1.5 – 2.0. Avoid to prepare stocks from not dense enough or old stationary phase cultures; ref. to (5). To reactivate, thaw the stocks slowly on ice or defrost them quickly in warm water and transfer them right away into growth medium preincubated at 26°C (with selective antibiotic - if recombinant strain) in ventilated TC flasks. Use the **entire content of the vial at once** for 10 ml medium and do not refreeze the glycerol stocks. Usually, you see motile cells immediately after inoculation. After 2 - 3 days the cultures from the first inoculation must be diluted 1:5 to 1:10 into fresh medium. Don't keep the first inoculation culture from glycerol stocks longer than 4 days before passaging.

(9) Do not use the first inoculation culture from glycerol stocks immediately for transfection. Passage the culture at least one - two times, better more often before electroporation (ref. to the description in the LEXSY expression kit manuals).

(10) Always control appearance and motility of cells by microscopy. Cells of mid growth phase cultures are of drop like shape approx. 15 x 5 µm in size with one flagellum at the flat end and motile. **These cells are most efficient for transfection, plating on solid media and preparation of glycerol stocks**. Mid growth phase cultures always contain subpopulations of not 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 midgrowth culture stage. Also, bacterial, fungal or other contaminations may be identified by microscopy.

(11) Keep patient, esp. if you are used to work with bacteria. *Leishmania* cells are protozoans with regular doubling times of 7 h in static suspension cultures and 5 h in agitated cultures. They need their time to grow or to adapt to new conditions and sometimes they seem to be a bit inactively. Continuous inoculations into fresh medium, regular resuspension of sedimented cells in static suspension cultures in ventilated TC flasks (6), a dark place and some calm and they will recover faster than you think.

(12) If you - despite following these instructions - eventually should encounter growth problems with the host strain, sediment cells 5 min at 2000g, resuspend pellet carefully in fresh growth medium and continue incubation in ventilated cell culture flasks. This approach was very helpful in rescuing cultures.

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

(14) Nobody is perfect. Please, don't hesitate to contact the Jena Bioscience Team if you encounter problems with cultivation or for further questions.