Preparation of 100 ml LEXSY BHI agar (4 plates)

Component	Storage	Amount / 100 ml
• Autoclave or melt (microwave) **50 ml 2% BACTO-Agar** and keep at 55°C	RT	50 ml
• Prepare **50 ml plating medium**:		
2x LEXSY BHI **(Cat. No. ML-412)**	RT	35 ml
inactivated (20 min 56°C) FCS	-20°C	10 ml
1M HEPES, pH 7.4	4°C	4 ml
Pen/Strep **(Cat. No. ML-105)**	-20°C	1 ml
Hemin (0,25% in 50% Triethanolamin) **(Cat. No. ML-108)**	4°C	0.2 ml
Antibiotic(s), if necessary		

- Pour the medium to the warm agar, mix gently and distribute 25 ml per plate with serological pipette; AVOID AIR BUBBLES!
- Dry the plates after solidifying for **exactly 10 min open** under the Air flow box
- If appropriate, cover agar surface with nitrocellulose membrane*
- Plate the freshly prepared plates immediately, at least on the same day
- Spread carefully 50-100 µl concentrated cells from 2 ml o/n culture after electroporation or 10^3 cells of an established suspension cultures per plate (plating efficiency is approx. 10%)
- To avoid “swarming” of *Leishmania* cells let the plates eventually open after plating until liquid film is evaporated (2-5 min)
- **Seal with Parafilm** and incubate at 26°C bottom up
- Colonies will start to appear usually after 5-7 days

* Nitrocellulose membrane (BA-85 0.45 µm blotting grade) allows transfer of clones to different cultivation conditions (e.g. induction of T7-TR driven expression) and makes plating easier.

The LEXSY plating kit (Cat. No. ML-451) contains all components for preparation of 40 plates for clonal selection of LEXSY expression strains.