Sibley Lab
8/29/2006
MM/LDS

Phelomycin BLE Selection

Following electroporation of desired plasmid, grow parasites for 2-3 day incubation (for RH88) in a T25 flask containing HFF cells.

1. Scrape and harvest 1- T25 of freshly egressed parasites.
2. Syringe 2x@ 20g, needle (optional).
3. Filter using 3 micron filter and HHE wash (HBSS + 10 mM HEPES + 0.1 mM EGTA).
4. Spin 1800 rpm 12" at room temperature.
5. Resuspend in HHE and count.
6. Prepare D0% (DMEM + 10mM Hepes + gentamycin) to pH 7.6 and keep in CO_2 incubator to maintain pH until needed.

Selection:

Resuspend parasites to 10^7 cells/ml D0%:
(place in incubator 15" with loose caps to stabilize pH)
To 1 ml or 2 ml of resuspended cells add 10 μl/ml PHLEOMYCIN stock
STOCK (made in DPBS + Ca) = 5 mg/ml FINAL CONC = 50 μg/ml.

Incubate toxo in 15ml PS tubes with caps very loose in 37°C CO_2 incubator 4 hours.

After 4 hours; centrifuge 1800rpm 12" to pellet. Resuspend pellet back to 10^7cells/ml with DMEM 10% FBS + 5 μg/ml phleo.

Inoculate HFF cultures in T25 flasks (approx. 10^7 parasites). A second round of selection should be performed before subcloning. Subclone by limiting dilution in 96 well plates containing HFF monolayers and select with 5 ug/ml of phelo.

NOTE OF SPECIAL IMPORTANCE:
THE pH OF DMEM 0% DURING DRUG SELECTION IS CRITICAL! ALKALINE pH WILL GREATLY ENHANCE THE TOXICITY OF THE PHLEOMYCIN.

Phleomycin drug: ordered from CAYLA company

Make stock at 5 mg/ml in PBS, store as aliquots at -70C. The solution should be pale blue. Contact:

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