Tetracycline Protocol and Selection Guide

Background

Tetracycline is a bacteriostatic polyketide antibiotic frequently used in a wide range of *in vitro* cell culture applications**.** It is most commonly used in tetracycline controlled gene expression systems (gene switches) such a the tet-on and tet-off systems. Recommended for use in cell culture applications at 10 mg/L.

Preparation and storage

Stock solution:

1. Tetracycline is kept in the 4C fridge in 68-564D. It is light sensitive.
2. Weigh 400mg of tetracyline HCL into a small weigh boat.
3. Dilute 95% Ethanol to 70% using milliQ water.
   a) Adding 20ml of milliQ to 60ml of 95% ethanol gives 80ml of 71% ethanol.
4. Add 80ml of 70% Ethanol to a 250ml bottle.
5. Add the tetracycline HCL to the ethanol.
6. Mix/vortex vigorously so all the tetracycline goes into solution.
7. Filter sterilize the solution into a falcon tube using a 20ml syringe and a 200nm filter.
8. Aliquot into pcr tubes and 1.7ml eppendorfs.
9. Store at -20C and protect any unused stock solution from light.
10. Store the small aliquots in the small box and the big aliquots in the larger box.

Mammalian Cell Culture

Transfection and Drug Selection

1. Grow cells to ~80% confluence in complete medium and transfect your plasmid with appropriate method.

2. After 24-48 hours of transfection, cells are split to 1:10, 1:20 or 1:50 into (2) 15 cm plates containing 25 ml of DMEM + 10% FBS + appropriate concentration of drug. Leftovers of transfected cells can be plated in 10cm plates containing 10 ml media. If you use Tet-OFF system, add 5 µg/ml of tetracycline or 1 µg/ml of doxycycline to shut off the protein expression.

3. Observe cell growth in every 2-3 days and change medium with selection drug every week or more if necessary. After 2–4 weeks, isolated colonies should begin to appear

Competent Cell Protocol

1. Streak out frozen stock of XL1- Blue MRF' bacteria on an LB-tet plate. Grow overnight at 37 degrees C.

2. Inoculate a single colony into a 250 ml flask containing:20 ml of SOC 20ul of 12.5 mg/ml tetracycline

3. Grow overnight in a shaker at 37 degrees C.

4. The next day set up two 2 liter flasks containing 250 ml 2XYT. Into each flask, pipet 2.5 ml of overnight culture.

5. Grow with shaking until culture reaches an OD600 of ~0.5.

6. Set up two centrifuge bottles on wet ice and transfer culture to bottles by pouring.

7. Pellet bacteria by spinning in Sorvall centrifuge in GSA rotor; 5000 r.p.m., 4 degrees C., 10 minutes.

8. Working in the cold room, with cells on ice asmuch as possible: Pour out supernatant, then use pipet to remove all residual fluid.

9. Resuspend each pellet gently in 83 ml RF1. Use 10 ml pipet to gently pipet up and down until all clumps have been resuspended.

10. Incubate on wet ice in cold room for 1 hour.

11. Pellet bacteria by spinning in Sorvall centrifuge in GSA rotor; 5000 r.p.m., 4 degrees C., 10 minutes.

12. Working in the cold room, with cells on ice as much as possible: Pour out supernatant, then use pipet to remove all residual fluid.

13. Resuspend each pellet gently in 20 ml of RF2. Use 10 ml pipet to gently pipet up and down until all clumps have been resuspended.

14. Incubate on wet ice in cold room for 15 minutes.

15. Take microcentrifuge tubes out of –80 degree C freezer and at least 160 tubes with open lids in an ice bucket containing wet ice. Dispense 100 ul of cell suspension into each tube. If possible, work with a second person, one person dispensing the cells, and the second person sealing tubes as they are filled. Once tubes are sealed, drop them in a Dewar flask containing liquid nitrogen. Once all 40 ml of competent cells are dispensed into microcentrifuge tubes, freeze all remaining tubes in liquid nitrogen. Collect tubes and store in a paper freezer box with no dividers.

16. Measure the efficiency of the competent cells by transforming a standard amount of commercial plasmid. Express efficiency as colony forming units / microgram of DNA [cfu/ug]. If you transform 0.1 ng of plasmid DNA and obtain 500 colonies, the efficiency of the cells is 5 x 10 6cfu/ug.

General Selection Table

Cell-Line	Species	Tissue	Media	Tetracycline
AGS	Human	stomach; gastric adenocarcinoma	RPMI	1 (µg/ml)
Caco-2	Human	Colon; colorectal adenocarcinoma	DMEM	1 (µg/ml)
CL1-5			RPMI	1 (µg/ml)
HeLa S3	Human	Cervix; adenocarcinoma		1 (µg/ml)
LL/2 (LLC1)	Mouse	lung; Lewis lung carcinoma	RPMI	1 (µg/ml)
MDA-MB-	Human	pleural effusion (metastasis);	DMEM	1 (µg/ml)

231		adenocarcinoma (mammary gland primary)		
Rcho-1			NCTC-135 medium	1 (µg/ml)
RKO	Human	Colon; carcinoma; control for RKO-E6 and RKO-AS45-1 cells		1 (µg/ml)
TA3/St			DMEM	1 (µg/ml)

References:

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