

1. Materials that you received.

#RF-5 : BW25113 RFzero-iy

Genotype: *prf4::zeo*, BAC8(cat), piodoTyrRS-MJR1-gent(pACYC)

Antibiotics: Cm 17 mg/L, Gent 7 mg/L, Zeo 25 mg/L

3-bromo/chloro/iodotyrosine: 0.1mg/ml

2. Medium used

The stub medium (1 mL) contains agar (1.5mg), NaCl (1mg), Yeast Extracts (0.25mg), Tryptone (0.5mg), and 3-bromotyrosine (0.1 mg).

The shipped stub was inoculated with a culture incubated in LB containing 0.1 mg/mL bromotyrosine, Cm 17 mg/L, Gent 7 mg/L, Zeo 25 mg/L.

3. How to supplement 3-bromo/chloro/iodotyrosine in the media

(1) 3-halogenated tyrosines can be dissolved in 0.2N HCl at a conc. of 10mg/mL (100 × final conc.)

(2) No sterilization is necessary for the solution.

(3) Shelf life of the solution is a month at 4 degrees.

(4) Add to the growth media and then neutralize with 2N NaOH just before the media preparation.

(5) Antibiotics are not necessary to maintain strains, and may be used to avoid contamination by other lab bacteria.

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