

## Library Storage Medium

### Recipe:

Put some 100 ml of water and a stirring rod into a measuring cylinder, and then weigh in:

10.0 g Tryptone  
5.0 g Yeast Extract  
5.0 g Sodium Chloride  
(OR 20.0 g premixed LB Lennox)  
101 g Glycerol

Fill up to 900 ml with water, adjust pH to 7.5 (RT, NaOH), adjust volume to 1000 ml, and autoclave in a Schott bottle. Once cooled, add 2.0 ml of a sterile  $50 \text{ g} \cdot \text{l}^{-1}$  carbenicillin stock and 250  $\mu\text{l}$  of purchased  $100 \text{ g} \cdot \text{l}^{-1}$  Zeocin.

### Library Storage Medium:

LB Lennox, 8% (v/v) glycerol, pH  $7.5 \pm 0.2$  (NaOH) at 25 °C plus  $100 \mu\text{g} \cdot \text{ml}^{-1}$  carbenicillin,  $25 \mu\text{g} \cdot \text{ml}^{-1}$  zeocin

### LB Lennox:

$10.0 \text{ g} \cdot \text{l}^{-1}$  Tryptone,  $5.0 \text{ g} \cdot \text{l}^{-1}$  Yeast Extract,  $5.0 \text{ g} \cdot \text{l}^{-1}$  Sodium Chloride, pH  $7.0 \pm 0.2$  (NaOH) at 25 °C or purchased ready made

### Carbenicillin stock (50 g · l-1):

Dissolve 5 g of carbenicillin in 50 ml of sterile water, washing the glass vial multiple times. Dilute to a total volume of 100 ml, filter-sterilize into two 50 ml conical tubes and store at -20°C or colder. Use 2.0 ml for 1 litre of storage medium ( $100 \mu\text{g} \cdot \text{ml}^{-1}$ ).

### Zeocin stock:

Zeocin is delivered in liquid form at  $100 \text{ g} \cdot \text{l}^{-1}$ . Use 250  $\mu\text{l}$  for 1 litre of storage medium ( $25 \mu\text{g} \cdot \text{ml}^{-1}$ ).

### Notes:

- Zeocin is inhibited by high ionic strength and at non-neutral pH. A pH of 7.5 is recommended by Invitrogen, a pH of 7.0 is recommended by OpenBiosystems. The use of LB Lennox, i.e. half the usual amount of NaCl is highly recommended by both. These limitations apply only to long-term storage, not plasmid cultures.
- 101 g of glycerol corresponds to 80 ml ( $\sigma = 1.262 \text{ kg} \cdot \text{l}^{-1}$ )