

## PREPARATION OF RETROVIRAL REAGENTS

### Media

#### 1. RV Media

DMEM, 10% FBS, 1x Glucose, 1x P/S

#### 2. 100x Glucose (3.5 mg/ml)

70 g sucrose (RT shelf)  
Bring final volume to 200 ml with QH<sub>2</sub>O.  
Filter through a 0.2  $\mu$ m filter.  
Aliquot into 50 ml tubes.  
Store at RT in tissue culture room.

#### 3. 25 mM chloroquine

MW= 515.9 g/mole (RT shelf)  
Weigh out 0.129 g. Add 10 ml PBS.  
Filter through a 0.2  $\mu$ m filter. Aliquot in 1.5 ml microcentrifuge tubes.  
Store at -20C.

### Transfection Reagents

#### 1. 100X Polybrene ( 0.8 mg/ml)

Powder is in 4C dessicator.  
Weigh out 0.08 g polybrene.  
Add 100 ml PBS.  
Filter through a 0.2  $\mu$ m filter. Aliquot into 5 ml tubes.  
Store at -20C.

#### 2. 2X BES (50 mM BES, 280 mM NaCl, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>)

Add BES, pH to 7.05  $\pm$  0.05  
Add NaCl and Na<sub>2</sub>HPO<sub>4</sub>

For 100 ml (use QH<sub>2</sub>O to bring up to volume):  
1.07 g BES (Sigma B-6137; MW= 213.2 g/mole; RT chest)  
1.64 g NaCl (MW= 58.44 g/mole)  
0.022 g Na<sub>2</sub>HPO<sub>4</sub> (MW= 141.96)

Filter through a 0.2  $\mu$ m filter. Aliquot into 10 ml tubes.  
Store at  $-20^{\circ}\text{C}$ . Avoid multiple freeze/thaw cycles.  
Use within 6 months.

### **3. 2M $\text{CaCl}_2$**

MW= 147.02 g/mole

Weigh out 7.35 g  $\text{CaCl}_2$  . Bring up to 25 ml with  $\text{QH}_2\text{O}$ .

Filter through a 0.2  $\mu$ m filter. Aliquot in 1.5 ml microcentrifuge tubes.

Store at  $-20^{\circ}\text{C}$ .