Nucleus Protocols

# Make Small Molecule Mix

## 1. Prepare Folinic Acid Stock (5 mM).

- ☐ Weigh 12.5 mg folinic acid.
- ☐ Dissolve to a final volume of 4.89 mL.
- ☐ Aliquot and freeze at –20°C.

## 2. Make All Other Stock Solutions.

- ☐ Use the table below to prepare the specified stock solutions:

| Reagent | MW (g/mol) | Amount (g) | Final Volume (mL) | Storage | Needs pH adjustment? | Needs Sterilization? |
|---|---|---|---|---|---|---|
| Potassium Hydroxide (1 M) | 56.11 | 14.0 | 250 | 4°C to 30°C | no | no |
| HEPES-KOH (pH 7.6, 1 M) | 238.3 | 59.5 | 250 | 4°C to 30°C; dark | yes | no |
| Potassium glutamate (2.5 M) | 203.23 | 21.8 | 50 | 4°C to 30°C | no | no |
| Magnesium acetate (1 M) | 214.45 | 10.8 | 50 | 4°C to 30°C | no | no |
| Creatine phosphate (500 mM) | 327.14 | 1 | 9.43 | –25°C to –15°C | no | no |
| Folinic acid (5 mM) | 511.50 | See above | | –25°C to –15°C | no | no |
| Spermidine (500 mM) | 145.25 | 1 | 13.77 | –25°C to –15°C | no | no |
| Amino Acid Mix | | | see Make Amino Acid Mix | –85°C to –15°C | yes | yes |

## 3. Assemble Small Molecule Mix Components.

- ☐ Use the table below to combine the previously prepared stock solutions into Small Molecule Mix:

| Reagent | Stock Concentration (mM) | Concentration in Small Molecule Mix (mM) | Volume to Add (uL) |
|---|---|---|---|
| HEPES-KOH (pH 7.6) | 1000 | 125 | 62.5 |
| Potassium glutamate | 2500 | 250 | 50.0 |
| Magnesium acetate | 1000 | 18.75 | 9.38 |
| rATP | 100 | 5 | 25.0 |
| rGTP | 100 | 5 | 25.0 |

| Reagent | Stock Concentration (mM) | Concentration in Small Molecule Mix (mM) | Volume to Add (uL) |
|---|---|---|---|
| rCTP | 100 | 2.5 | 12.5 |
| rUTP | 100 | 2.5 | 12.5 |
| Creatine phosphate | 500 | 50 | 50.0 |
| TCEP | 500 | 2.5 | 2.5 |
| Folinic acid | 5 | 0.05 | 5.0 |
| Spermidine | 500 | 5 | 5.0 |
| Amino Acid Mix | 3.25 | 0.75 | 115.4 |
| Ultrapure water | n/a | n/a | 15.82 |
| **Total** | | | 500 |

## 4. Storage

- ☐ Aliquot Small Molecule Mix into 1.5 mL microfuge tubes (between 50 µL and 100 µL per aliquot) and store at –80°C.