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	166.67	83.33
Potassium glutamate	2500	333.33	66.67
Magnesium acetate	1000	25	12.5
rATP	100	6.67	33.33
rGTP	100	6.67	33.33

Reagent	Stock Concentration (mM)	Concentration in Small Molecule Mix (mM)	Volume to Add (uL)
rCTP	100	3.33	16.67
rUTP	100	3.33	16.67
Creatine phosphate	500	66.67	66.67
TCEP	500	3.33	3.33
Folinic acid	5	0.067	6.67
Spermidine	500	6.67	6.67
Amino Acid Mix	3.25	1.00	153.85
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.