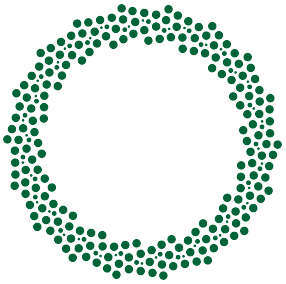

Nucleus Protocols

Make Small Molecule Mix

1. Protocol

- **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 −20C.
- **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	4C to 30C	no	no
HEPES-KOH (pH 7.6, 1 M)	238.3	59.5	250	4C to 30C; dark	yes	no
Potassium glutamate (2.5 M)	203.23	21.8	50	4C to 30C	no	no
Magnesium acetate (1 M)	214.45	10.8	50	4C to 30C	no	no
Creatine phosphate (500 mM)	327.14	1	9.43	−25C to −15C	no	no
Folinic acid (5 mM)	511.50	See above		−25C to −15C	no	no
Spermidine (500 mM)	145.25	1	13.77	−25C to −15C	no	no
Amino Acid Mix			see Make Amino Acid Mix	−85C to −15C	yes	yes

- **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

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

1.1. Storage

- Aliquot Small Molecule Mix into 1.5 mL microfuge tubes (between 50 uL and 100 uL per aliquot) and store at −80C.