



**Nucleus
Protocols**

Make OnePot Protein Mix — Bill of Materials

Name	Category	Product	Manufacturer	Part #	Price	Storage	Link
HEPES	Salts	HEPES, Crystalline Powder, ≥99.5% (titration), Poly bottle	Sigma-Aldrich	H3375-500G	\$431	4°C to 30°C	link
Magnesium chloride	Salts	Magnesium chloride hexahydrate, BioXtra, ≥99.0%	Sigma-Aldrich	M2670-500G	\$115.00	4°C to 30°C	link
Potassium chloride	Salts	Potassium Chloride, ACS reagent, 99.0-100.5%	Sigma-Aldrich	P3911-1KG	\$146.00	4°C to 30°C	link
Ammonium chloride	Salts	Ammonium chloride, ACS reagent, ≥99.5%	Sigma-Aldrich	213330-500G	\$73.50	4°C to 30°C	link
Potassium hydroxide	Salts	Potassium hydroxide - ACS reagent, ≥85%, pellets	Sigma-Aldrich	221473-1KG	\$99.90	4°C to 30°C	link
Imidazole	Salts	ReagentPlus® Sigma-Imidazole, Crystalline, 99%	Sigma-Aldrich	I202-500G	\$120	4°C to 30°C	link
Potassium glutamate	Salts	L- Glutamic acid potassium monohydrate	Sigma-Aldrich	G1501-100G	\$74.80	4°C to 30°C	link
Magnesium acetate	Salts	Magnesium acetate tetrahydrate	Sigma-Aldrich	M0631-100G	\$34.80	4°C to 30°C	link
EDTA	Salts	Ethylenediaminetetraacetic acid, BioUltra, ≥99.0% (KT)	Sigma-Aldrich	03609-250G	\$139.00	4°C to 30°C	link
Glycerol	Supplements	Glycerol, Molecular Biology Grade, Liquid, ≥99.0%	Sigma-Aldrich	G5516-1L	\$157.00	4°C to 30°C	link

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TCEP	Reagent	Thermo Scientific, Bond-Breaker® Tris[2-Carboxyethyl]phosphine Neutral Solution (TCEP), Application=Water-Soluble Reducing Agent, Molecular Weight=286.65, Size=5 mL	Thermo Scientific	77720	\$175.65	4°C to 30°C	link
Creatine phosphate	Reagent	Sodium creatine phosphate dibasic tetrahydrate, ≥98.0%(NT)	Sigma-Aldrich	27920-1G	\$102	-25°C to -15°C	link
Folinic acid	Reagent	Folinic acid calcium salt hydrate	Sigma-Aldrich	F7878-100M	\$108	4°C to 30°C	link
Spermidine	Reagent	Spermidine, ≥99% (GC)	Sigma-Aldrich	S2626-5G	\$176	-25°C to -15°C	link
Amino acids	Reagent	L-Amino acids, analytical standard	Sigma-Aldrich	LAA21-1KT	\$558	1°C to 4°C	link
IPTG	Media	Isopropyl β-D-thiogalactoside (IPTG), Powder, ≥99% (TLC), ≤0.1% Dioxane	Sigma-Aldrich	I6758-1G	\$89.90	-25°C to -15°C	link
BL21(DE3) Competent Cells	Biologic	BL21(DE3) Competent <i>E. coli</i>	NEB	C2527H	\$252	-85°C to -75°C	link
RNase Inhibitor, Murine	Reagent	RNase Inhibitor, Murine	NEB	M0314S	\$87.00	-25°C to -15°C	link
PURExpress Kit	Reagent	PURExpress® <i>In Vitro</i> Protein Synthesis Kit	NEB	E6800L	\$2774.00	-85°C to -75°C	link

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Nucleus PURE DNA constructs	Biologic	Pooled PURE expression constructs (see Make 36-pot)	b.next	—	—	-25°C to -15°C	DNA/PURE/expression
pOpen-deGFP DNA template	Biologic	deGFP reporter template (see Reporter deGFP)	b.next	—	—	-25°C to -15°C	pOpen-deGFP.gbk
<i>E. coli</i> Ribosomes	Biologic	Nucleus 70S ribosomes (see Make Ribosomes)	b.next	—	—	-80°C	Make Ribosomes
<i>E. coli</i> tRNAs	Biologic	Nucleus Total tRNA (see Make tRNAs)	b.next	—	—	-80°C	Make tRNAs
96-well Polypropylene DeepWell Plates	Consumables	Nunc™ 96-Well Polypropylene DeepWell™ Sample Processing & Storage Plates with Shared-Wall Technology	Nunc	260251	\$379.61	4°C to 30°C	link
Amicon Ultra 0.5 mL - 3 kDa	Consumables	Amicon® Ultra Centrifugal Filter, 3 kDa MWCO	Millipore Sigma	UFC5003	\$63	4°C to 30°C	link
Amicon Ultra 15 mL - 3 kDa	Consumables	Amicon® Ultra Centrifugal Filter, 3 kDa MWCO	Millipore Sigma	UFC9003	\$125	4°C to 30°C	link
Breathe-Easy sealing membrane	Consumables	Breathe-Easy® sealing membrane	Sigma-Aldrich	Z380059-1PA	\$96.50	4°C to 30°C	link

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Columns	Columns	Empty Disposable PD-10 Columns	Cytiva	17043501	\$258.30	4°C to 30°C	link
Ni-His Resin	Resin	NEBExpress Ni Resin	New England Biolabs	S1428S	\$358.00	1°C to 4°C	link
Rapid-Flow Sterile Single Use Vacuum Filter Units	Consumables	Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters (PES, 0.22 µm)	Thermo Scientific	596-3320	\$105.97	4°C to 30°C	link
SealPlate film	Consumables	SealPlate® film	Excel Scientific	Z369659-1000	\$72.20	4°C to 30°C	link