Nucleus Protocols

Prepare Protein Purification Buffers and Media

1. Step 1: Prepare Stock Buffers and Media

☐ Make or buy the following stock solutions. Use ultrapure water (18.2 MΩ, e.g., Milli-Q).

Stocks	Final Concentration (mM)	MW (g/mol)	Mass to add (g)	Final Vol (mL)	Storage (°C)
HEPES-KOH (pH 7.6)	1000	238.3	238.3	1000	room temp
Ammonium Chloride	1000	53.49	53.49	1000	room temp
Sodium Chloride	5000	58.44	58.44	1000	room temp
Potassium Chloride	1000	74.55	74.55	1000	room temp
Magnesium Chloride	1000	203.3	203.3	1000	room temp
Imidazole-HCl	1000	68.08	68.08	1000	room temp
Lysozyme	30 mg/mL	☐	0.3	10	–20°C
LB	1x	☐	25	1000	room temp
Kanamycin	50 mg/mL (1000x)	☐	2.5	50	–20°C
IPTG	500	238.3	238.3	50	–20°C

☐ Adjust pH of HEPES and Imidazole with Potassium Hydroxide and Hydrochloric Acid, respectively.

2. Step 2: Prepare Stable Buffers

☐ **Wash Buffer** — used to equilibrate columns and wash samples. Contains a small amount of Imidazole (20 mM) to reduce nonspecific protein binding to columns, improving purity.

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH (pH 7.6)	50	1000	100
Ammonium Chloride	100	1000	200
Sodium Chloride	500	5000	200
Magnesium Chloride	10	1000	20
Imidazole-HCl	20	1000	40
TCEP	1	500	4
Ultrapure water	—	—	1436
Total			**2000**

- ☐ **Elution Buffer** — used to elute proteins from Ni columns by introducing a large amount of Imidazole (500 mM) that competes with polyhistidine purification tags to bind the column.

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH (pH 7.6)	50	1000	12.5
Ammonium Chloride	100	1000	25
Sodium Chloride	500	5000	25
Magnesium Chloride	10	1000	2.5
Imidazole-HCl	500	1000	125
TCEP	1	500	0.5
Ultrapure water	—	—	59.5
Total			**250**

- ☐ **Protein Buffer** — used to remove Na+ and Imidazole from proteins. Filter with a 0.22 µm vacuum filter.

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH (pH 7.6)	50	1000	25
Potassium Chloride	100	1000	50
Magnesium Chloride	10	1000	5
TCEP	1	500	1
Ultrapure water	—	—	419
Total			**500**

- ☐ **Protein Buffer (60% glycerol)** — added to samples in Protein Buffer as a cryoprotectant for storage at –80°C. Filter with a 0.22 µm syringe filter. To use, add an equal volume of 60% glycerol buffer to samples in Protein Buffer (1:1).

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH (pH 7.6)	50	1000	2.5
Potassium Chloride	100	1000	5
Magnesium Chloride	10	1000	0.5
TCEP	1	500	0.1
glycerol	60%	100%	30
ultrapure water	—	—	11.9
Total			**50**

3. Step 3: Prepare Working Buffers

- ☐ **Lysis Buffer** — used to resuspend bacterial pellets for lysis. Also enzymatically lyses cells with lysozyme (*G. gallus*). Contains protease inhibitor (cOmplete) to slow proteolysis.

Reagent	Final Concentration (mM)	Stock Concentration (mM)	Volume to Add (mL)
HEPES-KOH (pH 7.6)	50	1000	5
Ammonium Chloride	100	1000	10
Sodium Chloride	500	5000	10
Magnesium Chloride	10	1000	1
Lysozyme	0.3 mg/mL	30 mg/mL	1
cOmplete Protease Inhibitor	1 tablet / 50 mL	n/a	2 tablets
TCEP	1	500	0.2
Ultrapure water	—	—	72.8
Total			**100**