

Instructions how to use template

1. Prepare Stock Solutions: Fill water to 75% of total volume → Weigh & dissolve required mass of salt (ensure MW of salt, may change depending on hydrated state) → Adjust pH (if listed, base= KOH, acid = HCl)→ Fill water to final volume.

2. Prepare Buffer Solution: Add required volume of each stock solution adjust to final volume with water → Omit TCEP until day of

3. Usage Notes: Modify highlighted values in blue → Check units carefully → Output values shown in purple.

Stock Solutions	MW (g/mol)	Concentration (M)	Volume (L)	Mass (g)
Ammonium Chloride	53.49	1	1	53.49
HEPES-KOH pH 7.6	238.3	1	1	238.3
Imidazole-HCl pH 7	68.08	1	1	68.08
Magnesium Chloride	95.21	1	0.25	23.80
Potassium Chloride	74.55	1	1	74.55
Sodium Chloride	58.44	5	1	292.2
Glycerol		100%		
TCEP		0.5		

	Lysis Buffer		Wash Buffer		Elution Buffer		Protein Buffer (4x)	
Solutions	Concentration (mM)	Volume required (mL)	Concentration (mM)	Volume required (mL)	Concentration (mM)	Volume required (mL)	Concentration (mM)	Volume required (mL)
Ammonium Chloride	100	70	100	200	100	100		
HEPES-KOH pH 7.6	50	35	50	100	50	50	200	200
Imidazole-HCl pH 7			20	40	500	500		
Magnesium Chloride	10	7	10	20	10	10	40	40
Potassium Chloride							400	400
Sodium Chloride	500	70	500	200	500	100		
TCEP	1	1.4	1	4.000	1	2.000		
Water		516.6		1436		238		360
	Total Volume (mL)	700	Total Volume (mL)	2000	Total Volume (mL)	1000	Total Volume (mL)	1000

Protein stock buffer (4x) will be used to make dialysis and stroager buffers (60%, 30%). The difference between the three buffers are the amount of glycerol.

	Dialysis Buffer		Storage Supplement Buffer (60% gly)		Storage Buffer (30% gly)	
Solution	Concentration	Volume Required (mL)	Concentration	Volume Required (mL)	Concentration	Volume Required (mL)
Protein stock buffer (4x)	1	10	1	11.25	1	2.5
Glycerol			60	27	30	3
TCEP	1	0.08	2	0.18	1	0.020
Water		29.92		6.57		4.48
	Total Volume (mL)	40	Total Volume (mL)	45	Total Volume (mL)	10