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Buffer Recipes for Protein Purification

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Equilibration buffer

1

25 mM HEPES, 100 mM NaCl, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	1.461 g	2.922 g	5.844 g	11.688 g	23.376 g

Dissolve in water; bring pH up to 7.5; store at 4°C

25 mM HEPES, 500 mM NaCl, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.88 g

Dissolve in water; bring pH up to 7.5; store at 4°C

Lysis buffer

2

50 mM Na/K phosphate buffer, 500 mM NaCl, 2.5% glycerol, pH 8.0

	250 mL	500 mL	1 L	2 L	4 L
NaH ₂ PO ₄ • H ₂ O (MW=137.9923)	0.0690 g	0.1380 g	0.2760 g	0.5520 g	1.1039 g
K ₂ HPO ₄ (MW=174.18)	2.0902 g	4.1803 g	8.3607 g	16.7213 g	33.4426 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.88 g
glycerol	6.25 mL	12.5 mL	25 mL	50 mL	100 mL

Dissolve in water; bring pH up to 8.0; store at 4°C

Wash buffer

3

25 mM HEPES, 1M NaCl, 10 mM imidazole, 10% glycerol, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	14.61 g	29.22 g	58.44 g	116.88 g	233.76 g
imidazole (MW=68.08)	0.1702 g	0.3404 g	0.6808 g	1.3616 g	2.7232 g
glycerol	25 mL	50 mL	100 mL	200 mL	400 mL

Dissolve in water; bring pH up to 7.5; store at 4°C

25 mM HEPES, 1M NaCl, 10 mM imidazole, 10% glycerol, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	14.61 g	29.22 g	58.44 g	116.88 g	233.76 g
imidazole (MW=68.08)	0.3404 g	0.6808 g	1.3616 g	2.7232 g	5.4464 g
glycerol	25 mL	50 mL	100 mL	200 mL	400 mL

Dissolve in water; bring pH up to 7.5; store at 4°C

Elution buffer

4 25 mM HEPES, 100 mM NaCl, 300 mM imidazole, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	1.461 g	2.922 g	5.844 g	11.688 g	23.376 g
imidazole (MW=68.08)	5.106 g	10.212 g	20.424 g	40.848 g	81.696 g

Dissolve in water; bring pH up to 7.5; store at 4°C

25 mM HEPES, 500 mM NaCl, 300 mM imidazole, pH 7.5

	250 mL	500 mL	1 L	2 L	4 L
HEPES (MW=238.31)	1.4894 g	2.9789 g	5.9578 g	11.9155 g	23.831 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.88 g
imidazole (MW=68.08)	5.106 g	10.212 g	20.424 g	40.848 g	81.696 g

Dissolve in water; bring pH up to 7.5; store at 4°C



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