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## Buffer Recipes for Protein Purification

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** Buffers, protein purification

## Equilibration buffer

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25 mM HEPES, 100 mM NaCl, pH 7.5

	250 m L	500 m L	1 L	2 L	4 L
HEPES (MW=238.31)	1.489 4 g	2.978 9 g	5.957 8 g	11.91 55 g	23.83 1 g
NaCl (MW=58.44)	1.461 g	2.922 g	5.844 g	11.68 8 g	23.37 6 g

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

25 mM HEPES, 500 mM NaCl, pH 7.5

	250 m L	500 m L	1 L	2 L	4 L
HEPES (MW=238.31)	1.489 4 g	2.978 9 g	5.957 8 g	11.91 55 g	23.83 1 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.8 8 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 m L	500 m L	1 L	2 L	4 L
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O (MW=137.99 23)	0.069 0 g	0.138 0 g	0.276 0 g	0.552 0 g	1.103 9 g
K <sub>2</sub> HPO <sub>4</sub> (MW=174.18)	2.090 2 g	4.180 3 g	8.360 7 g	16.72 13 g	33.44 26 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.8 8 g
glycerol	6.25 mL	12.5 mL	25 mL	50 mL	100 m L

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 m L	500 m L	1 L	2 L	4 L
HEPES (MW=238.31)	1.489 4 g	2.978 9 g	5.957 8 g	11.91 55 g	23.83 1 g
NaCl (MW=58.44)	14.61 g	29.22 g	58.44 g	116.8 8 g	233.7 6 g
imidazole (MW=68.08)	0.170 2 g	0.340 4 g	0.680 8 g	1.361 6 g	2.723 2 g
glycerol	25 mL	50 mL	100 m L	200 m L	400 m L

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 m L	500 m L	1 L	2 L	4 L
HEPES (MW=238.31)	1.489 4 g	2.978 9 g	5.957 8 g	11.91 55 g	23.83 1 g
NaCl (MW=58.44)	14.61 g	29.22 g	58.44 g	116.8 8 g	233.7 6 g
imidazole (MW=68.08)	0.340 4 g	0.680 8 g	1.361 6 g	2.723 2 g	5.446 4 g
glycerol	25 mL	50 mL	100 m L	200 m L	400 m L

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 m L	500 m L	1 L	2 L	4 L
HEPES (MW=238.31)	1.489 4 g	2.978 9 g	5.957 8 g	11.91 55 g	23.83 1 g
NaCl (MW=58.44)	1.461 g	2.922 g	5.844 g	11.68 8 g	23.37 6 g

imidazole (MW=68.08)	5.106 g	10.21 g	20.42 g	40.84 g	81.69 g
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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)	1.489 g	2.978 g	5.957 g	11.91 g	23.83 g
NaCl (MW=58.44)	7.305 g	14.61 g	29.22 g	58.44 g	116.8 g
imidazole (MW=68.08)	5.106 g	10.21 g	20.42 g	40.84 g	81.69 g

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