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Ready-to-Use His Buffer Kit

The Ready-to-Use His Buffer kit contains concentrated stock solutions for time-saving, reproducible and convenient purification operation.

Each kit contains 2 x 50 ml stock phosphate buffer together with NaCl (0.16 M phosphate , 4 M NaCl, pH 7.4) and 50 ml stock imidazole solution (2 M, pH 7.4).

Binding buffer/sample is recommended as: 20 mM sodium phosphate + 0.5 M NaCl, pH 7.4. For the purification of his-tagged proteins, the presence of a low concentration of imidazole is recommended. The exact concentration is protein and metal ion dependent with a guided range of 10 – 50 mM.

Washing buffer can be the same as the binding buffer or may contain imidazole of a higher concentration (e.g. 50 mM).

Elution solution is recommended as 250 mM – 500 mM of imidazole in 20 mM phosphate buffer + 0.5 M NaCl, pH 7.4.

The standard buffer condition is:

- Binding buffer / sample: 20 mM imidazole in 20 mM phosphate + 0.5 M NaCl, pH7.4
- Washing buffer: 20 mM imidazole in 20 mM phosphate + 0.5 M NaCl, pH7.4
- Elution buffer: 500 mM imidazole in 20 mM phosphate + 0.5 M NaCl, pH7.4

Table 1: Mixing table for Standard Binding buffer / sample (10 ml)

	Case - 1	Case - 2	Case - 3
Cell lysate (pellet)	0 ml	5 ml	8 ml
Stock phosphate buffer	1.25 ml	1.25 ml	1.25 ml
Stock imidazole solution	0.1 ml	0.1 ml	0.1 ml
Distilled water	8.65 ml	3.65 ml	0.65 ml
Total	10 ml	10 ml	10 ml

Table 2: Mixing table for Standard Washing buffer and Elution buffer (50 ml)

	Stock phosphate buffer	Stock imidazole solution	Distilled water	Final imidazole concentration in buffer
Washing buffer	6.25 ml	0.5 ml	43.25 ml	20 mM
Elution Buffer	6.25 ml	12.5 ml	31.25 ml	500 mM

Table 3: Mixing table for 50 ml buffer of varied imidazole concentration

Imidazole concentration in buffer	Stock phosphate buffer	Stock imidazole solution	Distilled water
0 mM	6.25 ml	0 ml	43.75 ml
10 mM	6.25 ml	0.25 ml	43.50 ml
20 mM	6.25 ml	0.50 ml	43.25 ml
30 mM	6.25 ml	0.75 ml	43.00 ml
40 mM	6.25 ml	1.00 ml	42.75 ml
50 mM	6.25 ml	1.25 ml	42.50 ml
60 mM	6.25 ml	1.50 ml	42.25 ml
70 mM	6.25 ml	1.75 ml	42.00 ml
80 mM	6.25 ml	2.00 ml	41.75 ml
90 mM	6.25 ml	2.25 ml	41.50 ml
100 mM	6.25 ml	2.50 ml	41.25 ml
150 mM	6.25 ml	3.75 ml	40.00 ml
200 mM	6.25 ml	5.00 ml	38.75 ml
250 mM	6.25 ml	6.25 ml	37.50 ml
300 mM	6.25 ml	7.50 ml	36.25 ml
400 mM	6.25 ml	10.00 ml	33.75 ml
500 mM	6.25 ml	12.50 ml	31.25 ml

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The prepared buffers above all contain 20 mM Phosphate + 0.5 M NaCl, pH7.4. If the required buffer volume is different from the volume listed above, the recipe components will be changed proportionally.

Ordering information

Product	Quantity	Code no.
Ready-to-Use His Buffer Kit	2 x 50 ml phosphate stock solution (0.16 M sodium phosphate + 4 M NaCl, pH 7.4) and 50 ml imidazole stock solution (2 M, pH7.4)	200105
Related products	Quantity	Code no.
Ni SepFast	10 ml	180113
	25 ml	180104
	100 ml	180105
Pre-packed Ni SepFast column	1 x 1 ml	180114
	1 x 5 ml	180116
Cu SepFast	25 ml	180106
	100 ml	180107
Co SepFast	25 ml	180108
	100 ml	180109
Zn SepFast	25 ml	180110
	100 ml	180111
Screening kit of IMAC SepFast	5 ml each of Ni SepFast, Co SepFast and Zn SepFast, respectively; plus 1 Ready-to-Use His Buffer Kit	180103
IMAC SepFast (non-charged format)	10 ml	180112
	25 ml	180101
	100 ml	180102
Ni SuperSpin	50	150101
Co SuperSpin	50	150103
Zn SuperSpin	50	150104
Ni SepFast MAG	5 ml	190101
Co SepFast MAG	5 ml	190103
Zn SepFast MAG	5 ml	190104

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