

# Geneflow

## Ready-to-Use His Buffer Kit Instructions

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 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 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**

<b>Imidazole Concentration in Buffer</b>	<b>Stock Phosphate Buffer</b>	<b>Stock imidazole Solution</b>	<b>Distilled Water</b>
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

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	Geneflow Product No.	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)	P7-0014	200105
Related products	Quantity	Geneflow Product No.	Code No.
Ni SepFast BG	10 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0024	180113
	25 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0026	180104
	100 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0028	180105
Cu SepFast BG	25 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0030	180106
	100 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0032	180107
Co SepFast BG	25 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0034	180108
	100 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0036	180109
Zn SepFast BG	25 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0038	180110
	100 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0040	180111
Screening kit of IMAC SepFast BG	5 ml each of Ni SepFast BG, Cu SepFast BG, Co SepFast BG and Zn SepFast BG, respectively; plus 8 columns (4 BG-30 and 4 BG-5), four 10 ml syringes, 1 Ready-to-Use His Buffer Kit	P7-0048	180103
IMAC SepFast BG (non-charged format)	10 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0042	180112
	25 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0044	180101
	100 ml, BG-30 and BG-5 columns and 10 ml syringe (one each)	P7-0046	180102
Ni SuperSpin	50	P7-0006	150101
Cu SuperSpin	50	P7-0008	150102
Co SuperSpin	50	P7-0010	150103
Zn SuperSpin	50	P7-0012	150104

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