Instructions 28-4010-39 AC

His Buffer Kit

Intended use

His Buffer Kit is intended for research use only, and should not be used in any clinical or in vitro procedures for diagnostic purposes.

His Buffer Kit contains

- Phosphate buffer 8 x stock solution, pH 7.4 (160 mM sodium phosphate, 4 M NaCl). Volume 2 x 100 ml.
- 2 M imidazole, pH 7.4. Volume 1 x 100 ml.
- Instructions for use.

Purpose

His Buffer Kit contains stock solutions of binding and elution buffers for purification of histidine-tagged proteins. The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient purification work.

His Buffer Kit can be used in combination with a number of column types, such as His SpinTrap™, His MultiTrap™ FF, His MultiTrap HP, His GraviTrap™, HisTrap™ FF, HisTrap FF crude and HisTrap HP.

Table of contents

1. Principle 2

2. Buffer preparation 3

3. Optimization of imidazole concentration 5

4. Purification protocol 6

5. Ordering information 6

1 Principle

Imidazole is commonly used to elute histidine-tagged proteins from IMAC (immobilized metal affinity chromatography) media.

To minimize the binding of unwanted host cell proteins, imidazole is also used at a low concentration in the binding buffer. For this reason, it is important to include imidazole in the sample at the same concentration as in the binding buffer.

20 mM concentration of imidazole in the binding buffer commonly gives good purification results but the purification is protein-dependent. Optimization of the imidazole concentration may be required, see Section 3.

2 Buffer preparation

The choice of optimal imidazole concentration in the binding buffer is a balance between purity and yield. The recommended imidazole concentrations are 20 mM in the binding buffer and 500 mM in the elution buffer. Table 1 specifies volumes of supplied phosphate buffer and imidazole solution required to prepare binding buffer and elution buffer for various columns.

Table 1. Preparation of binding buffer and elution buffer for different columns[1].

Column	Buffer	Phosphate buffer stock solution (ml)	Imidazole 2M (ml)	Dest. water	Imidazole final conc. (mM)
His SpinTrap (10X)	Binding	2.5	0.2	to 20 ml	20
	Elution	0.75	1.5	to 6 ml	500
His GraviTrap	Binding	3.75	0.3	to 30 ml	20
	Elution	1.0	2.0	to 8 ml	500
HisTrap 1 ml	Binding	3.75	0.3	to 30 ml	20
	Elution	1.0	2.0	to 8 ml	500
HisTrap 5 ml	Binding	16.25	1.3	to 130 ml	20
	Elution	5.0	10.0	to 40 ml	500
His MultiTrap (one 96-well plate)	Binding	27.5	2.2	to 220 ml	20
	Elution	10.0	20.0	to 80 ml	500

[1] His Buffer Kit can also be used for batch/gravity flow purification using Ni Sepharose 6 Fast Flow medium and empty disposable PD-10 columns.

For preparing binding buffer and elution buffer with other concentrations of imidazole, see Table 2. The prepared buffers will contain 20 mM sodium phosphate, 0.5 M NaCl and the chosen imidazole concentration at pH 7.4

Table 2. Preparation of binding buffer and elution buffer with different imidazole concentrations.

Imidazole final conc. (mM)	Phosphate buffer stock solution (ml)	Imidazole 2M (ml)	Dest. water
0	6.25	0.00	to 50 ml
10	6.25	0.25	to 50 ml
20	6.25	0.50	to 50 ml
40	6.25	1.00	to 50 ml
60	6.25	1.50	to 50 ml
80	6.25	2.00	to 50 ml
100	6.25	2.50	to 50 ml
500	6.25	12.50	to 50 ml

When preparing buffers please note that:

- always include imidazole in the sample at the same concentration as the binding buffer.
- formation of salt crystals may occur in the stock solutions at low temperatures. To avoid salt crystals, let the stock solutions adjust to room temperature before use.

3 Optimization of imidazole concentration in buffers

Binding buffer

The choice of optimal imidazole concentration in the binding buffer is a balance between purity and yield. The highest purity is obtained using the imidazole concentration of the step before the histidine-tagged protein elutes, see below.

To optimize the imidazole concentration in the binding buffer the following procedure can be performed:

1 Prepare a number of buffers with increasing concentrations of imidazole, for example 10, 20, 40, 60 and 500 mM (Table 2).

2 Equilibrate the column with 10 column volumes binding buffer containing the lowest selected concentration (i.e. 10 mM imidazole).

3 Apply the sample and collect the flow-through fraction.

4 Wash with 15 column volumes binding buffer and collect the wash fractions.

5 Add 5 column volumes buffer containing the next higher concentration of imidazole and collect the flow-through fraction.

6 Continue stepwise elution with 5 column volumes of higher and higher imidazole concentrations. Collect fractions from each step.

7 Determine purity of the collected fractions, for example by SDS-PAGE analysis.

Note: High concentrations of imidazole in the binding buffer will prevent the binding of histidine-tagged proteins.

Elution buffer

The optimum imidazole concentration in the elution buffer is the one that elutes the histidine-tagged protein completely. 500 mM imidazole can be used for elution in most cases.

4 Purification protocol

Purification protocol for the protein purification procedure is found in the instructions for the selected column/media or at www.gelifesciences.com/trap and www.gelifesciences.com/hitrap.

5 Ordering information

Product	Pack size	Code No.
His Buffer Kit	1	11-0034-00
His GraviTrap kit[1]	1	28-4013-51
His SpinTrap kit[2]	1	28-9321-71

1 Includes 2 packs His GraviTrap and 1 pack His Buffer Kit
2 Includes 1 pack His SpinTrap and 1 pack His Buffer Kit

Related products	Pack size	Code No.
His GraviTrap	10 × 1 ml	11-0033-99
His MultiTrap FF	4 x prepacked 96-well plates	28-4009-90
His MultiTrap HP	4 x prepacked 96-well plates	28-4009-89
His SpinTrap	50 × 100 µl	28-4013-53
HisTrap FF, 1 ml	5 × 1 ml	17-5319-01
HisTrap FF, 5 ml	5 × 5 ml	17-5255-01
HisTrap FF crude, 1 ml	5 × 1 ml	11-0004-58
HisTrap FF crude, 5 ml	5 × 5 ml	17-5286-01
HisTrap HP, 1 ml	5 × 1 ml	17-5247-01
HisTrap HP, 5 ml	1 × 5 ml	17-5248-01
HisTrap HP, 5 ml	5 × 5 ml	17-5248-02
Ni Sepharose 6 Fast Flow	5 ml	17-5318-06
Ni Sepharose 6 Fast Flow	25 ml	17-5318-01
Ni Sepharose 6 Fast Flow	100 ml	17-5318-02
Ni Sepharose High Performance	25 ml	17-5268-01

Related products	Pack size	Code No.
Ni Sepharose High Performance	100 ml	17-5268-02
Empty Disposable PD-10 columns	50	17-0435-01
LabMate PD-10 Buffer Reservoir	10	18-3216-03

Literature	Code No.
Ni Sepharose and IMAC Sepharose, Selection guide	28-4070-92
Affinity Chromatography Columns and Media, Selection guide	18-1121-86
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Recombinant Protein Purification Handbook	18-1142-75

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