

QuickPick™ IMAC Plus

62321 • metal affinity kit for proteins, 8 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ IMAC kit. Please read the entire instructions carefully before starting to work with the reagents. The QuickPick IMAC reagents are intended for use with the QuicPick™ one magnet magnetic tool supplied by BN Products & Services. Also refer to the QuicPick one magnet instructions for use. The QuickPick IMAC kit provides a fast and simple means for recombinant histidine-tagged (His-tag) protein purification based on metal affinity using Ni²⁺.

SPECIFICATIONS

Recommended vessel format:	Volumes used in this kit vary from 400 µl to 3 ml. Microtubes and for example 12-well plates, small glass beakers (25 ml) etc. are recommended.
Yield per preparation*:	Up to 0.5 mg (His-tagged glutathione-S-transferase)
Sample volume:	Cells from 10 - 40 ml of culture medium are suspended in 3 ml of Wash Buffer 1. The amount of culture required depends on the level at which the protein is expressed.
Elution volume:	400 µl
Total protocol time*:	≥ 10 minutes

* Yield and total protocol time depend on binding time used. See figure 1 on page 3.

KIT CONTENTS

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IMAC Magnetic Particles	6.6 ml
IMAC Regeneration Buffer	24 ml
IMAC ^{5x} Wash Stock Buffer*	21 ml
IMAC Imidazole Buffer, 500 mM	3.5 ml
IMAC Elution Buffer	4.0 ml
QuicPick tips	8 pcs

IMAC Magnetic Particles:	IMAC magnetic particles in phosphate buffer (pH 7.0), NaCl, Tween 20, 0.02 % NaN ₃
IMAC Regeneration Buffer:	Aqueous NiSO ₄ solution, Tween 20, 0.02 % NaN ₃
IMAC ^{5x} Wash Stock Buffer*:	Phosphate buffer (pH 7.0), NaCl, Tween 20, 0.1 % NaN ₃
IMAC Imidazole Buffer, 500 mM:	500 mM imidazole in phosphate buffer (pH 7.0), 0.02 % NaN ₃
IMAC Elution Buffer:	20 mM Tris-HCl (pH 8.0), 250 mM NaCl, 300 mM imidazole, 0.05 % Tween 20, 0.02 % NaN ₃

* IMAC ^{5x} Wash Stock Buffer is used for the preparation of IMAC Wash Buffers 1, 2 and 3.

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. QuicPick one magnet magnetic tool. See also the QuicPick one magnet instructions for use.
2. Microtubes and appropriate reaction vessels.
3. QuicPick tip box for storing the QuicPick tips.

PRINCIPLE OF THE METHOD

QuickPick IMAC Magnetic Particles are formed from magnetic agarose particles which provide an extensive porous surface area especially suitable for the purification of His-tag proteins from cell lysates. The transfer of magnetic particles with QuicPick technology enables rapid purification of His-tag recombinant proteins. The methodology removes the need of using time-consuming column technology.

Immobilized metal ion affinity chromatography (IMAC) was originally introduced as a method for group separation, but today it represents one of the most important tools for the single-step purification of proteins based on polyhistidine tags. The principle of the mechanism is based on the interaction between a metal ion coordinated to a covalently bound chelating ligand and a His-tag protein.

PROCEDURE

QuicPick tips

The QuicPick tips are ready to use. The tips are not sterile, but can be autoclaved (+121 °C at least 20 min) provided that they are first removed from the bag. The separately available QuicPick tip box can also be autoclaved.

The following notes are important for the procedure:

1. To avoid foaming, only mild pulse vortexing is recommended for all solutions (contain Tween 20).
2. Mix the IMAC Magnetic Particles suspension thoroughly before pipetting into microtubes.
3. Between 2 to 4 ml of resuspended cell material can be used as sample.
4. The volume of IMAC Elution Buffer may be between 300-600 µl depending on the protein concentration required for the downstream application.
5. If parallel reactions are performed simultaneously with QuicPick one magnet the next reaction may be started while the first sample is being incubated with magnetic particles in the sample tube. The QuicPick tip of the first reaction can be stored in an extra tube or in the QuicPick tip box during the preparation of the next reaction. Remember to mix the sample tube of the first reaction occasionally.
6. To decrease the unspecific adsorption of contaminating proteins, 3 ml of IMAC Wash Buffer 3 can be used in an optional wash step before the elution step. See section: “**Preparing IMAC Wash Buffers 1, 2 and 3**”.
7. In order to obtain higher yields the sample incubation time in step 2 of the protocol can be increased as shown in Fig.1.

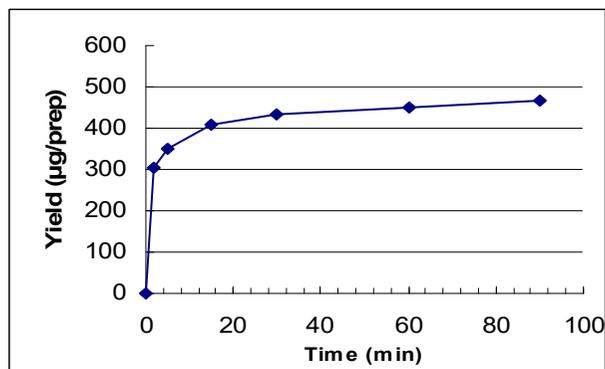


Figure 1: The effect of binding time on yield

Sample preparation

IMAC Wash Buffer 1 is used to resuspend the pelleted cells. To avoid unspecific protein binding in the sample, the concentration of NaCl may be adjusted up to 1 M. Also, imidazole may be added up to a concentration of 5 mM. Add protease inhibitors, protein stabilizers or other additives into Wash buffer 1, if needed.

The most commonly used methods for disruption of bacterial cells are ultrasonication and French press.

Example: For one preparation thoroughly resuspend *E. coli* cell pellet (from 20 ml fermentation) into 3 ml of IMAC Wash Buffer 1. Sonicate the suspension on an ice-bath with 10-15 x 10 s pulses. Leave a 10 s interval without sonication between each pulse to prevent warming of the suspension. After sonication, centrifuge the suspension for 10 minutes at 18 000 x g and use the supernatant as the sample. (If lower centrifugation speed is used the centrifugation time should be increased, for example 20 minutes at 10 000 x g).

The amount of sample required depends on the level at which the protein is expressed, which must be determined empirically for each expression experiment. If the protein is not expressed efficiently, cells from a larger culture volume should be used.

Preparing IMAC Wash Buffers 1, 2 and 3

IMAC Wash Buffer 1

Prepare by diluting IMAC ^{5x} Wash Stock Buffer in distilled water as follows. For example, for one reaction pipet:

$$\begin{array}{r} 600 \mu\text{l IMAC}^{5x} \text{ Wash Stock Buffer} \\ \underline{2400 \mu\text{l H}_2\text{O}} \\ \text{Final volume} = 3000 \mu\text{l} \end{array}$$

IMAC Wash Buffer 2

Prepare by diluting IMAC ^{5x} Wash Stock Buffer in distilled water and add IMAC Imidazole Buffer, 500 mM to the solution. For example, for one reaction pipet:

$$\begin{array}{r} 600 \mu\text{l IMAC}^{5x} \text{ Wash Stock Buffer} \\ 120 \mu\text{l IMAC Imidazole Buffer, 500 mM} \\ \underline{2280 \mu\text{l H}_2\text{O}} \\ \text{Final volume} = 3000 \mu\text{l} \end{array}$$

The amounts of imidazole and NaCl in the final buffer are 20 mM and 250 mM, respectively.

IMAC Wash Buffer 3 (optional)

To decrease the unspecific adsorption of contaminating proteins 0.2-1.0 M NaCl and 5-40 mM imidazole can be used in an optional wash step. For example, for one reaction pipet:

600 μ l IMAC ^{5x} Wash Stock Buffer
240 μ l IMAC Imidazole Buffer, 500 mM
450 μ l 5 M NaCl (not included in the kit)
1710 μ l H₂O

Final volume = 3000 μ l

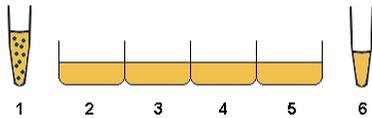
The amounts of imidazole and NaCl in the final buffer are 40 mM and 1000 mM, respectively.

QuickPick™ IMAC kit protocol with QuicPick one magnet

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Make sure that Wash Buffers 1, 2 and 3 (optional) are correctly prepared from the IMAC ^{5x} Wash Stock Buffer before continuing. IMAC Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles.

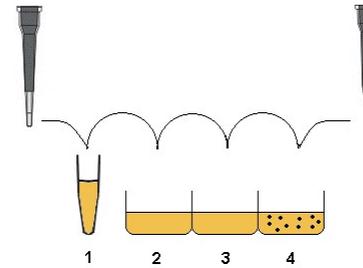
Number tubes/vessels from 1 to 6 and pipette liquids before starting as follows:

- 1: 750 μ l IMAC Magnetic Particles
- 2: 3 ml IMAC Regeneration Buffer
- 3: 3 ml IMAC Wash Buffer 1
- 4: 3 ml sample (see “**Sample preparation**”)
- 5: 3 ml IMAC Wash Buffer 2
- 6: 400 μ l IMAC Elution Buffer

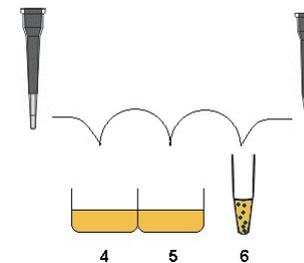


Protocol:

1. Pick up the QuicPick tip using the QuicPick one magnet. Collect the magnetic particles from tube 1 (Magnetic Particles) with QuicPick and release them into vessel 2 (Regeneration Buffer). If the magnetic particles have formed clumps, they can be broken up by using the QuicPick tip. Mix the suspension briefly and gently using the QuicPick tip. Note that the magnet has to be withdrawn at this point.
2. Collect the magnetic particles from vessel 2 and release them in vessel 3 (Wash Buffer 1). Suspend the magnetic particles thoroughly with the QuicPick tip and mix briefly and gently. Transfer the magnetic particles from vessel 3 into vessel 4 (sample). Mix them thoroughly into the solution and incubate at room temperature for 5-90 minutes (See figure 1 on page 3 for time-course data on yield). Mix the solution continuously to avoid sedimentation.

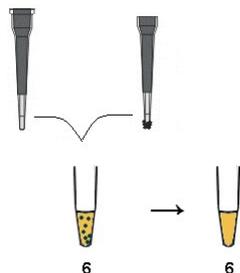


3. Collect the magnetic particles from vessel 4 and wash them in vessel 5 (Wash Buffer 2) by releasing the magnetic particles into the solution and mixing gently for 10 s. An optional wash step may be carried out similarly with Wash Buffer 3 to decrease unspecific protein binding. Collect the magnetic particles from vessel 5 and release them into tube 6 (Elution Buffer). Mix the magnetic particles thoroughly in the solution and incubate for at least 1 minute. Mix the solution continuously to avoid sedimentation.



Incubate for 1 minute

4. After incubation collect the magnetic particles from tube 6 and discard them and the tip. The purified His-tagged protein is ready to be used in downstream applications.



Collect the magnetic particles and discard them.

STORAGE AND STABILITY

The QuickPick IMAC Plus kit should be stored at +2°-+8°C. Magnetic particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick IMAC Plus kit is intended for research use only, and not intended for use in human diagnostic or therapeutic procedures. All solutions contain 0.02-0.04 % sodium azide (NaN₃) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN₃ are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions. The product contains nickel sulphate (NiSO₄) which is harmful if ingested, inhaled or absorbed through skin contact. Nickel sulphate is a possible carcinogen and teratogen.

DISCLAIMERS AND WARRANTIES

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