



GENETIX BRAND

HANDBOOK



Nucleopore[®] His-Spin Protein Miniprep

Nucleopore[®] His-Spin Protein Miniprep NP- 1002 P 10 Preps



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COMPONENTS

Kit contents

Nucleopore® His-Spin Protein Miniprep

Cat #	NP-1002P (10 preps.)
Affinity Gel HAG	1.4 ml X 2
Binding Buffer HBB	10 ml
Wash Buffer HWB	10 ml
Elution Buffer HEB	5 ml
Spin columns	10
Collection tubes	10
Handbook	1

*Please see "Preparation and Storage of reagents"

Reagents, consumables and equipments not provided with the kit

- Ethanol
- Pipette and Tips
- Centrifuge
- Vortexer

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS).

His-Spin Nickel Affinity Gel

Contains : N,N,-bis-(carboxymethyl)-L-Cysteine , Ethyl Alcohol & Nickel (II) sulfate hexahydrate
R&S Phrases: 10-20/21/22-40-42/43-51/53-68/20/21/22; S: 23-45-36/37-61

His-Wash Buffer (Component A)

Contains: Sodium chloride
R&S Phrases: S 24/25

His-Wash Buffer (Component C)

Contains: Triton X-45
R&S Phrases : R36/38, S26, S37

His-Elution Buffer (Component A)

Contains: Sodium chloride
R&S Phrases : S 24/25

His-Elution Buffer (Component C)

Contains: Triton X-45
R&S Phrases : R36/38, S26, S37

R10: Flammable, R20/21/22: Harmful by inhalation, in contact with skin and if swallowed, R36/38: Irritating to eyes and skin, S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, R40: Limited evidence of a carcinogenic effect, R42/43: May cause sensitization by inhalation and skin contact, R51/53: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment, R68/20/21/22: Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. S23: Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer), S24/25: Avoid any inhalation, contact with skin and eyes. Wear suitable protective clothing and gloves, S37: Wear suitable gloves, S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible), S36/37: Wear suitable protective clothing and gloves, S61: Avoid release to the environment. Refer to special instructions/safety data sheet

INTRODUCTION

The Nucleopore His-Spin Protein Miniprep enables fast and convenient purification of His-tagged protein. The purification is based on a nickel-charged His-Affinity Gel (IMAC), and unique Spin column technology. Up to 1 mg of His-tagged protein can be eluted into 100 µl of the provided Elution Buffer HEB in 5 minutes. The purified protein can be used directly for enzymatic assays, protein biochemical analyses, SDS-PAGE, as well as other protein based applications.

Principle and Procedure

The kit utilizes specially formulated Buffer set combined with Spin column technology resulting in elution of high quality and pure protein suitable for enzymatic assays, protein biochemical analyses, SDS-PAGE and other applications

Specifications of Nucleopore® Cleanup Kit

Protein Purity – High-quality, purified Protein is eluted with Elution buffer and is especially well suited for enzymatic assays, protein biochemical analyses, SDS-PAGE and other applications..

Typically, up to 1 mg His tagged protein can be eluted in 100 µl of Elution Buffer HEB.

Preparation and Storage of reagents

The kit should be stored at 4 degree for 1 year. It can also be stored at room temperature without any effect on stability. Keep the buffers cold, or put them on ice before use for purification of sensitive proteins. The affinity gel may be kept at room temperature for up to 7 days.

Sample Preparation

Cells expressing the polyhistidine-tagged protein may be directly resuspended in the Binding Buffer HBB and lysed by standard methods including sonication, repeated freeze-thaw cycles, french press, etc. Any cell extract or other complex protein mixtures can be used as a starting material as long as the proteins are soluble. pH value of the loaded sample should be between 7.5 and 8.0 . For unknown proteins dilute the starting material with one volume of the Binding Buffer HBB before proceeding with the purification process.

PROTOCOL FOR HIS-TAGGED PROTEIN PURIFICATION

Things to do before starting

Refer to the instructions given in " Sample Preparation" section

Procedure

1. **Transfer 250ul of Affinity Gel HAG to Spin Column in a 2ml Collection Tube.** The resin should be fully resuspended by shaking/vortexing the bottle before pipetting
2. **Centrifuge for 5-10 seconds. Discard the flow-through.** Ensure that the Affinity Gel is completely drained. Do not over-dry the gel by long centrifugation times
3. **Add 150-300µl Protein sample and resuspend the gel by shaking or tapping the column.**
4. **Incubate it for 2 minutes and resuspend the gel a few more times** . If sample volume is larger than 200ul, an additional 1-2 minutes binding time may be needed to improve yields of purified protein.
5. **Centrifuge for 5-10 seconds. Discard the flow-through.**
6. **Add 250µl Wash Buffer HWB to the column and resuspend the gel. Centrifuge for 5-10 seconds.**
7. **Repeat the above wash step. Discard flow-through.**
8. **Place Spin column into a 1.5ml micro centrifuge tube. Add 150 ul of Elution Buffer HEB to the column and resuspend the gel. Centrifuge 5-10 seconds to elute the purified protein.** Elution volumes can be between 100-200 ul. 150 ul of Elution Buffer elutes virtually all the column-bound protein.

The eluate now contains the purified protein. The eluted protein is suitable for many applications. Store the purified protein at appropriate temperature

TROUBLESHOOTING GUIDE

Low Yield

Possible cause

- Diluted starting material

Suggestions

- Check that the adequate amount of sample is reloaded on to the column, an extra incubation time should be given to sample more than 200ul

Possible cause

- Protein folding may hinder binding of the hexahistidine tag to the Affinity Gel HAG.

Suggestions

- Lowering the imidazole concentration of the Wash Buffer HWB to 25 mM may increase yields.

Possible cause

- Protein is bound to the Affinity Gel HAG too tightly and can not be completely eluted with the supplied Elution Buffer HEB.

Suggestions

- A custom-made elution buffer containing 500 mM imidazole or 100 mM EDTA will help to solve this problem.

No purified protein recovered

Possible cause

- His-tag rendered inaccessible as a result of protein folding or Recombinant protein can also be insoluble as a result of over expression

Suggestions

- The protein can be purified at denaturing conditions Important: Urea decomposition in these buffers may shift pH upon storage – check and re-adjust before use. For improved stability, it is recommended to store these buffers at 4°C or make fresh.

Binding buffer: 8 M urea, 10 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 8.0. Adjust the pH by addition of concentrated sodium hydroxide.

Washing buffer: 8 M urea, 50 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 6.3. After dissolving the components the solution will be approximately pH 6.3.

Elution buffer: 8 M urea, 250 mM imidazole, 0.1 M sodium phosphate monobasic, 0.01 M Tris, pH 4.5. Adjust the pH by addition of concentrated hydrochloric acid.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
® DNASure Tissue Mini Kit	50 preps	NP-61305
® DNASure Plant Mini Kit	50 preps	NP-79105
® DNASure Plant Mini Kit	250 preps	NP-79107
® DNASure Plant Midi Kit	20 preps	NP-78153
® DNASure Plant Maxi Kit	10 preps	NP-78164
® DNASure Blood Mini Kit	50 preps	NP-61105
® DNASure Blood Mini Kit	250 preps	NP-61107
® DNASure Blood Midi Kit	20 preps	NP-61184
® DNASure Blood Maxi Kit	10 preps	NP-61193
® DNASure Blood FastPure Kit	50 preps	NP-62205
® DNASure Blood FastPure Kit	250 preps	NP-62207
® SureSpin Plasmid Mini Kit	50 preps	NP-37105
® SureSpin Plasmid Mini Kit	250 preps	NP-37107
® SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
® SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
® SureSpin Buffer Set*	1	37107-BS
® SurePrep Plasmid Mini Kit	20 preps	NP-15123
® SurePrep Plasmid Mini Kit	100 preps	NP-15125
® SurePrep Plasmid Midi Kit	20 preps	NP-15143
® SurePrep Plasmid Midi Kit	100 preps	NP-15145
® SurePrep Plasmid Maxi Kit	10 preps	NP-15161
® SurePrep Plasmid Maxi Kit	25 preps	NP-15162
® SurePrep Plasmid Mega Kit	5 preps	NP-15183
® SurePrep Plasmid Giga Kit	5 preps	NP-15191

*SureSpin® Buffer Set

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

**SureSpin® Buffer Set

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleopore® His-Spin Protein Miniprep Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleopore® His-Spin Protein Miniprep Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleopore® His-Spin Protein Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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