

# Detergent Clean-up Micro Kit

For use with P/Ns 1006090, 1006364 and 1006365

# ProteoSpin™

Silicon Carbide Technology



# Application Manual

## ProteoSpin™ Detergent Clean-up Micro Kit Benefits

Basic Features	Benefits
A complete kit for acidic and basic protein samples	The kit contains essential solutions for processing small detergent-containing samples from 2–50 µg of protein. Solutions for processing both acidic and basic proteins are included.
No detergent carryover	Unlike HPLC procedures, each sample is processed individually using its own spin tube providing no carryover or column bleed from sample to sample.
Usable with a variety of detergents	This kit can be used to remove detergents including SDS, Triton X-100, CHAPS, NP-40, and Tween 20.
Effective detergent removal	Removes more than 95% of detergent for many proteins, allowing tryptic digestion without residual detergents that interfere with trypsin digestion.
High recovery	Protein recoveries of 80% to 95% for many proteins.
Fast processing time	In contrast to longer dialysis procedures, the easy-to-use procedure allows processing up to 12 samples in only 20 minutes.

For more information visit: [www.mdscelex.com](http://www.mdscelex.com)



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# ProteoSpin Detergent Clean-up Micro Kit

For use with Product Numbers 1006090, 1006364, and 1006365

DETE-MAN3-0304



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# Introduction and Description

# 1

The ProteoSpin™ Detergent Clean-up Kit provides a fast and simple procedure for the removal of SDS and other detergents from protein samples. Detergents are extensively used to prepare protein samples. However, detergents must be removed prior to downstream analysis because of their undesirable effects. For example, detergents can often cause poor results in instruments such as mass spectrometers due to large numbers of contaminating peaks. Also, live cells are very sensitive to detergents so that processes such as microinjection of proteins into cells, protein immunization into laboratory animals, among others, require proteins free of detergents. Detergents also interfere with purification procedures such as chromatography and gel electrophoresis. In addition, they interfere with structural characterization techniques such as mass spectrometry and amino acid sequence analysis. Detergents are surface-active agents (surfactants) that contain both a hydrophobic portion and a hydrophilic portion. In aqueous solutions, detergents form stable structures called micelles with their hydrophilic portions facing the aqueous environment and their hydrophobic portions hidden in the core.

The charge of the hydrophilic portion enables the classification of detergents into four major groups. These groups are anionic, cationic, zwitterionic and non-ionic. Anionic and cationic detergents can be used for processes ranging from solubilizing membrane proteins under non-denaturing conditions, to the denaturing conditions of running SDS-PAGE. Common ionic detergents include SDS and sodium deoxycholate.

Zwitterionic detergents, such as CHAPS, protect the native state of the protein while preventing protein aggregation during purification procedures. While zwitterionic detergents

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## Introduction and Description

have low denaturing features, non-ionic detergents are non-denaturing. This makes non-ionic detergents suitable for solubilizing membrane proteins without altering biological activity by maintaining the secondary and tertiary structures of the protein. However, these non-ionic detergents, including NP-40, Triton X-100 and Tween 20, are less effective at disrupting protein aggregation.

Conventional methods for detergent removal include hydrophobic adsorption, gel chromatography, dialysis, ion-exchange chromatography and precipitation techniques. These methods are time-consuming and tedious, are often not applicable to small volumes of protein solutions, and may also result in the loss of the protein sample. The ProteoSpin Detergent Clean-up Kit offers an innovative and user-friendly method that removes greater than 95% of detergents (based on Triton X-100 and SDS measurements) from protein samples while maintaining high protein recovery.

The ProteoSpin Detergent Clean-up Kit uses silicon carbide (SiC) as a chromatography matrix. The SiC acts as the ion exchanger, and is able to effectively remove greater than 95% of detergents present in the sample both efficiently and with ease. The kit is able to remove all types of detergents including ionic, non-ionic and zwitterionic. Furthermore, both free and tightly bound detergents can be removed with the ProteoSpin Detergent Clean-up Kit.

## Introduction and Description

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### **Ion Exchange Chromatography**

In ion exchange chromatography, ions that are electrostatically bound to an insoluble and chemically inert matrix are reversibly replaced by ions in solution. The matrix can be either an anion exchanger, consisting of positively charged groups that reversibly bind anions in solution, or it can be a cation exchanger bearing negatively charged groups that bind to mobile cations. Proteins are complex molecules that can have an overall positive or negative charge, depending on their amino acid composition and the pH of their solutions. In this case, any given protein will bind to both anion and cation exchangers depending on the net charge of the protein.

When purifying a protein, the pH and salt concentration of the buffer solution (in which the protein is dissolved) are chosen to immobilize the desired protein on the selected ion exchanger. The impure protein solution is applied to a column that contains the ion exchanger, and has been washed and equilibrated with the buffer solution. The proteins bind to the matrix and are eluted by either changing the salt concentration or the pH.

### **Silicon Carbide as an Ion Exchanger**

The chromatographic resin used in the ProteoSpin Detergent Clean-up Kit consists of silicon carbide (SiC), a man-made material that is noted for its hardness (second only to diamond) and high resistance to chemical change. These properties make SiC highly suitable for spin column chromatography.

When processed appropriately, SiC becomes an effective cation exchanger for the purpose of purifying macromolecules. The surface of SiC is negatively charged and can bind positively charged macromolecules. SiC has poor affinity to monovalent and divalent cations, also making it an effective resin for the removal of salts.

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## Introduction and Description

Soluble proteins bind to SiC through the interaction between the positively charged side-groups on the polypeptide chain and the negative charges on the SiC surface. As a cation exchanger, SiC has an estimated effective  $pK_a \approx 2$  making the resin negatively charged over a wide range of pH. Therefore, with the exception of those peptides that are highly acidic, most soluble polypeptides can be bound to SiC.

## Removal of Detergents from Protein Solutions Using SiC

The ProteoSpin Detergent Clean-up Kit is designed to remove detergents in protein solutions either in their free form or bound form as when complexed with the protein. Detergent molecules that are free in the solution cannot bind to SiC if the detergent is negatively charged because it is repelled by the negative charges on the surface of SiC. Positively charged detergents, however, are expected to preferentially interact with SiC, causing proteins to bind poorly.

By virtue of the polar and nonpolar regions in a detergent molecule, detergents can partition in both hydrophobic and hydrophilic environments. Most detergents unbind from proteins in an aqueous environment in the presence of alcohols, freeing the proteins to interact with water molecules. If the solution is now allowed to move along a chromatographic column containing the SiC resin, the protein molecules bind to the resin while the detergent moves along with the liquid phase. The captured protein can then be eluted into a different buffer free of detergents.

## Introduction and Description

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### Isoelectric Point

Proteins generally contain a number of ionizable groups with a variety of  $pK_a$  values. Each protein has a particular pH where the negative charges on the molecule exactly balance the positive charges, therefore giving the protein no net charge. This is known as the isoelectric point, or  $pI$  of the protein.

The  $pI$  of a protein determines the ideal conditions for its purification by ion exchange chromatography. For example, based on  $pI$ , one can determine whether the pH of a binding buffer and an elution buffer are suitable for a particular protein. Isoelectric points for a number of proteins have already been determined. If the  $pI$  of a protein of interest is unknown, a theoretical  $pI$  can be determined using web-based tools found at the ExPASy site [http://us.expasy.org/tools/pi\\_tool.html](http://us.expasy.org/tools/pi_tool.html). Another useful  $pI$ -predictor method can be found at [http://www.ap.univ-mrs.fr/~nubim/d\\_abim/compo-p.html](http://www.ap.univ-mrs.fr/~nubim/d_abim/compo-p.html).

Introduction and Description

**Kit Components**

**ProteoSpin™ Detergent Clean-up Kit Components**

Component	ProteoSpin Detergent Clean-up Micro Kit, 25 Samples	ProteoSpin Detergent Clean-up Micro Kit, 50 Samples	ProteoSpin Detergent Clean-up Micro Kit, 250 Samples
	1006090	1006364	1006365
Column Activation and Wash Buffer (Acidic)	8 mL	15 mL	75 mL
Column Activation and Wash Buffer (Basic)	8 mL	15 mL	75 mL
pH Binding Buffer (Acidic)	4 mL	8 mL	40 mL
pH Binding Buffer (Basic)	4 mL	8 mL	40 mL
Elution Buffer	4 mL	8 mL	30 mL
Neutralizer	1 mL	1 mL	4 mL
ProteoSpin SiC:Spin columns (filled with 25 mg SiC) inserted in 2 mL collection tube	25	50	250

## Introduction and Description

### ProteoSpin™ Detergent Clean-up Kit Components (cont'd)

Component	ProteoSpin Detergent Clean-up Micro Kit, 25 Samples	ProteoSpin Detergent Clean-up Micro Kit, 50 Samples	ProteoSpin Detergent Clean-up Micro Kit, 250 Samples
Final Collection Tube, 1.7 mL	30	60	300
ProteoSpin Detergent Clean-up Micro Kit Application Manual	1	1	1
ProteoSpin Detergent Clean-up Micro Kit Protocol Card	1-Acidic Protocol 1-Basic Protocol	1-Acidic Protocol 1-Basic Protocol	1-Acidic Protocol 1-Basic Protocol

### Recommended Storage Conditions

For unopened solution containers, the reagents should remain stable for at least six months when stored at room temperature. Once opened, the solution should be stored at 4 °C when not in use. Some precipitation will occur with 4 °C storage. This precipitation should be dissolved with slight heating to room temperature before using.

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## Introduction and Description

### **Customer-Supplied Reagents and Equipment**

You must have the following in order to use the ProteoSpin Detergent Clean-up Kit:

- Benchtop microcentrifuge
- Micropipettors
- pH indicator paper
- Other optional elution buffers
- Isopropanol

# Procedures

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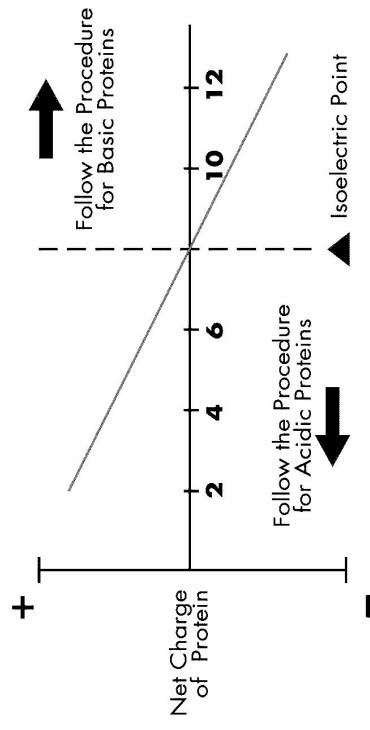
This section describes the procedures associated with the ProteoSpin™ Detergent Clean-up Micro Kit and how to select the appropriate one for your purposes.

## Choosing a ProteoSpin Detergent Clean-up Micro Kit Procedure

The ProteoSpin Detergent Clean-up Micro Kit comes with solutions for removing detergents from both acidic and basic proteins. In theory, the protocol for acidic proteins should apply to a majority of acidic and basic proteins since the resin is a cation exchanger; that is, all proteins with  $pI$  greater than the binding  $pH$  at 4.5 should bind. However, basic proteins bind strongly when they are used under these conditions, making their elution quite inefficient. Therefore, for soluble basic proteins ( $pI \geq 8$ ), a different condition for binding the protein to the resin has been developed. For the purposes of this kit, a protein whose  $pI$  is less than 8 will be treated as acidic and will use the acidic protocol; otherwise, use the basic protocol.

Procedures

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Choosing a procedure based on the isoelectric point

## Protocols

The following procedures describe the removal of detergents from acidic or basic proteins in either buffered or unbuffered (dissolved in water) solutions.

The kit utilizes spin columns containing SiC, which bind the protein of interest if it retains a net positive charge at the binding pH of 4.5. Non-specifically bound proteins and detergents are washed from the column and the specific protein is eluted into specially formulated elution buffer, removing the detergent from the protein sample. Each spin column will remove detergent from up to 50 ug of acidic or basic protein. This kit is designed to remove a variety of detergents and/or surfactants from protein solutions. It is capable of removing detergents such as Triton-X100, SDS, Chaps, NP-40 and Tween20. Each column is capable of processing up to 50 ug of protein, and will remove greater than 95% of detergents when present.

### Protocol for Acidic Proteins

Proteins with isoelectric points (*pI*) less than 7 are considered acidic. However, proteins with *pI* of less than 8 may be treated as acidic proteins when using this kit. If the *pI* of the protein being purified is not known, the theoretical *pI* may be calculated using the web-based applications at [https://us.expasy.org/tools/pi\\_tool.html](https://us.expasy.org/tools/pi_tool.html) or [https://www.uniprot-prot.org/~nabim/d\\_abim/compo-p.html](https://www.uniprot-prot.org/~nabim/d_abim/compo-p.html).

### Sample Preparation

The most critical steps in sample preparation are the proper pH adjustment of the solution to be applied to the column and the addition of isopropanol. The pH Binding Buffer (acidic) solution will be used to adjust the pH of your protein solution to 4.5.

1. Depending on your starting protein solution, you will require a certain volume of the pH Binding Buffer (acidic) to adjust the pH of your protein solution to 4.5. For example, if the starting protein solution is in water, add one (1) part of the pH Binding Buffer (acidic)

## Procedures

to 49 parts of the protein solution. If the starting protein solution is in a buffer, you may need greater volumes of the pH Binding Buffer (acidic) to lower the pH, depending on the buffer type and its ionic strength. The following table serves as a guideline for pH adjustment of protein solutions in buffers.

### pH Adjustment for Acidic Proteins

Starting pH of protein solution	Volume of pH Binding Buffer (acidic) to add per milliliter of protein solution for pH adjustment (based on 100 mM buffered solution)
5, 6, 7	20 $\mu$ L
8	50 $\mu$ L
9, 10, 11	80 $\mu$ L
12	100 $\mu$ L

**Note:** If your protein solution is already in a buffer and the pH is 4.5 or lower, you do not need to make any further adjustments.

2. Add one volume of isopropanol to the pH-adjusted solution.
3. Mix contents well and measure the pH before applying to the column.
4. Further adjust the pH if necessary.

## Procedures

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### Preparation of Modified Column Activation and Wash Buffer

This step prepares a modified solution to activate and wash the columns. It is prepared using the provided pH Binding Buffer (acidic) and isopropanol solutions.

1. Combine the following solutions in a sterile container (e.g., 50 mL conical tube), and mix well:
  - ◆ 0.5 mL pH Binding Buffer (acidic)
  - ◆ 12.5 mL isopropanol
  - ◆ 12.0 mL sterile deionized water
2. Label the container as Modified Column Activation and Wash Buffer (acidic) and keep it tightly sealed at room temperature when not in use. This volume is sufficient for the entire Detergent Clean-up Micro Kit.

### Column Activation

1. Open the cap on the pre-assembled spin column with its 2 mL collection tube.
2. Add 250  $\mu$ L of Modified Column Activation and Wash Buffer (acidic) to the column and close the cap.
3. Centrifuge for one minute, and discard the flowthrough.

**Note:** All centrifugation steps are carried out at 14,000  $\times g$  in a microcentrifuge.

Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4 °C, or on ice. Samples may be put on ice to preserve biological activity.

4. Repeat steps 2 and 3 to complete the column activation step.

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### Protein Binding

During the binding step, the protein solution is passed through the resin bed aided by centrifugation. The protein is captured as it comes in contact with the resin. For most proteins tested, the centrifugation time provides sufficient contact time for the protein to bind.

1. Apply a maximum of 1 mL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
2. Discard the flowthrough. (If desired, save the flowthrough in a fresh tube for assessing your protein's binding efficiency.) Reassemble the spin column with its collection tube.
3. Repeat steps 1 and 2 until entire protein sample has been applied to the column.
4. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

### Column Wash

This step removes non-specifically bound debris from the column.

1. Apply 250  $\mu$ L of Modified Column Activation and Wash Buffer (acidic) to the column and centrifuge for one minute.
2. Discard the flowthrough and reassemble the spin column with its collection tube.
3. Add 250  $\mu$ L of **regular** Column Activation and Wash Buffer (acidic) to the column and centrifuge for one minute.
4. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional minute to dry.

## Procedures

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### Protein Elution and pH Adjustment

The supplied Elution Buffer consists of 50 mM sodium phosphate pH 12.5. Consult Appendix A, (Optional Elution Buffers) for a list of alternate elution solutions that have been tested with the kit.

1. Add 5  $\mu\text{L}$  of Neutralizer to a fresh 1.7 mL microcentrifuge tube.
2. Transfer the spin column from the Column Wash procedure into the microcentrifuge tube.
3. Apply 25  $\mu\text{L}$  of the Elution Buffer to the column and centrifuge for one minute to elute the bound protein.
4. Add another 25  $\mu\text{L}$  of Elution Buffer and centrifuge for one minute in the same microcentrifuge tube.

**Note:** Approximately 95% of bound protein is recovered in the first two elutions. If desired, a third elution using 50  $\mu\text{L}$  of Elution Buffer may be carried out. This should be collected into a different tube (to which 5  $\mu\text{L}$  of Neutralizer is pre-added) to prevent dilution of the first two elutions.

Protein samples are now ready for downstream applications.

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## Procedures

### Protocol for Basic Proteins

Proteins with isoelectric points ( $pI$ ) greater than 7 are considered basic. However, proteins with  $pI$  greater than or equal to 8 may be treated as basic proteins when using this kit. If the  $pI$  of the protein being purified is not known, the theoretical  $pI$  may be calculated using the web-based application at [http://us.expasy.org/tools/pi\\_tool.html](http://us.expasy.org/tools/pi_tool.html) or [http://ca.expasy.org/tools/pi\\_tool.html](http://ca.expasy.org/tools/pi_tool.html), or [http://www.up.univ-mrs.fr/~nabim/d\\_abim/compo-p.html](http://www.up.univ-mrs.fr/~nabim/d_abim/compo-p.html).

### Sample Preparation

The most critical steps in sample preparation are the proper pH adjustment of the solution to be applied to the column and the addition of isopropanol. The pH Binding Buffer (basic) solution will be used to adjust the pH of your protein solution to 7.0.

1. Depending on your starting protein solution, you will require a certain volume of the pH Binding Buffer (basic) to adjust the pH of your protein solution to 7.0. For example, if the starting protein solution is in water, add one (1) part of the pH Binding Buffer (basic) to 49 parts of the protein solution. If the starting protein solution is in a buffer, you may need greater volumes of the pH Binding Buffer (basic) to lower the pH, depending on the buffer type and its ionic strength. The table below serves as a guideline for pH adjustment of protein solutions in buffers.

### pH Adjustment for Basic Proteins

Starting pH of protein solution	Volume of pH Binding Buffer (basic) to add per milliliter of protein solution for pH adjustment (based on 10 mM buffered solution)
4	150 $\mu$ L
5, 6	80 $\mu$ L
8, 9, 10	60 $\mu$ L
11, 12	60 $\mu$ L

2. Add one volume of isopropanol to the pH-adjusted solution.
3. Mix contents well and measure the pH before applying to the column.
4. Further adjust the pH if necessary.

### Preparation of Modified Column Activation and Wash Buffer

This step prepares a modified solution to activate and wash the columns. It is prepared using the provided pH Binding Buffer (basic) and isopropanol solutions

1. Combine the following in a sterile container (e.g, 50 mL conical tube), and mix well:
  - ◆ 0.5 mL pH Binding Buffer (basic)
  - ◆ 12.5 mL isopropanol
  - ◆ 12.0 mL sterile deionized water
2. Label container as Modified Column Activation and Wash Buffer (basic) and keep it tightly sealed at room temperature when not in use. This volume is sufficient for the entire detergent removal kit.

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## Procedures

### Column Activation

1. Open the cap on the pre-assembled spin column with its 2 mL collection tube.
2. Add 250  $\mu\text{L}$  of Modified Column Activation and Wash Buffer (basic) to the column and close the cap.
3. Centrifuge for one minute, and discard the flowthrough.

**Note:** All centrifugation steps are carried out at 14,000  $\times g$  in a microcentrifuge. Performance of the kit is not affected by temperature, and thus the procedure may be performed at room temperature, 4  $^{\circ}\text{C}$ , or on ice. Samples may be put on ice to preserve biological activity.

4. Repeat steps 2 and 3 to complete the column activation step.

### Protein Binding

During the binding step, the protein solution is passed through the resin bed aided by centrifugation. The protein is captured as it comes in contact with the SIC. For most proteins tested, the centrifugation time provides sufficient contact time for the protein to bind.

1. Apply a maximum of 1 mL of protein solution (from the Sample Preparation step) onto the column and centrifuge for one minute.
2. Discard the flowthrough. (If desired, save the flowthrough in a fresh tube for assessing your protein's binding efficiency.) Reassemble the spin column with its collection tube.
3. Repeat steps 1 and 2 until entire protein sample has been applied to the column.
4. Discard any remaining flowthrough and reassemble the spin column with its collection tube.

## Procedures

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### Column Wash

This step removes non-specifically bound debris from the column.

1. Apply 250  $\mu\text{L}$  of Modified Column Activation and Wash Buffer (basic) to the column and centrifuge for one minute.
2. Discard the flowthrough and reassemble the spin column with its collection tube.
3. Add 250  $\mu\text{L}$  of **regular** Column Activation and Wash Buffer (basic) to the column and centrifuge for one minute.
4. Inspect the column and ensure that the liquid has passed through into the collection tube. There should be no liquid in the column. If necessary, spin an additional minute to dry.

### Protein Elution and pH Adjustment

The supplied Elution Buffer consists of 50 mM sodium phosphate pH 12.5. Consult Appendix A, (Optional Elution Buffers) for a list of alternate elution solutions that have been tested with the kit.

1. Add 5  $\mu\text{L}$  of Neutralizer to a fresh 1.7 mL microcentrifuge tube.
2. Transfer the spin column from the Column Wash procedure into the microcentrifuge tube.
3. Apply 25  $\mu\text{L}$  of the Elution Buffer to the column and centrifuge for one minute to elute the bound protein.
4. Add another 25  $\mu\text{L}$  of Elution Buffer and centrifuge for one minute in the same microcentrifuge tube.

**Note:** Approximately 95% of bound protein is recovered in the first two elutions. If desired, a third elution using 50  $\mu\text{L}$  of Elution Buffer may be carried out. This should be collected into a different tube (to which 5  $\mu\text{L}$  of Neutralizer is pre-added) to prevent dilution of the first two elutions.

Protein samples are now ready for downstream applications.

Procedures

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# Optional Elution Solutions

# A

Proteins bound to SiC via interactions with electrostatic charges are eluted through pH-dependent mechanisms. The efficiency of protein elution depends on high pH above the *pI* of the protein to be purified. The pH of the elution buffer chosen must be at least one unit higher than the *pI* (isoelectric point) of the protein of interest. Solutions not provided with the ProteoSpin™ kit may be utilized if they are more appropriate for your needs. The table below describes optional elution buffers and their observed efficiency when BSA is used as test protein.

## Optional Elution Buffers

Volatiles Elution Buffers	Approximate Protein Recovery (based on 50 µg input BSA)
50 mM ammonium hydroxide (approximate pH 11)	70%
250 mM ammonium hydroxide (approximate pH 11)	70%
1 M ammonium hydroxide (approximate pH 11)	90%
1 M ethanolaniline (approximate pH 9)	70-80%

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Optional Elution Solutions

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Non-Volatile Elution Buffers	Approximate Protein Recovery (based on 50 µg input BSA)
50 mM sodium phosphate (approximate pH 12.5)	>95%
500 mM sodium phosphate (approximate pH 12.5)	<70%
100 mM sodium borate (approximate pH 12.5)	95-100%
1 M Tris (approximate pH 12.5)	95%

# Proteins with Established Isoelectric Points

# B

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Protein	Molecular Weight (kDa)	Isoelectric Point (pI)
Albumin, bovine serum	67	5.5
Albumin, human serum	66.5	4.8
Carbonic anhydrase	30	7.3
Carboxypeptidase	34	6.0
Catalase	250	5.6
Cytochrome C	13	10.6
Fibrinogen	330	5.5
Growth hormone, human	21.5	6.9
Hemoglobin, horse	65	6.9
Immunoglobulins G	150	6.4–7.2
Insulin	5.7	5.3
Lysozyme, hen egg white	14.3	11.0
Myoglobin, horse	17	7.0
Ovalbumin	40	4.6

Proteins with Established Isoelectric Points

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<b>Protein</b>	<b>Molecular Weight (kDa)</b>	<b>Isoelectric Point (pI)</b>
Pepsin	35.5	< 1.0
Ribonuclease	14	7.8
Thyroglobulin	660	4.6
Trypsin inhibitor, soybean	22.5	4.55
Urease	480	5.1

# Frequently Asked Questions and Troubleshooting



**What is the black material that is packed into the spin columns?** The black chromatography material is silicon carbide (SiC). It is processed using proprietary methods to function as an ion exchange chromatography resin.

**How does the kit remove detergents?** The Emulsifier causes the detergent to unbind from the protein and/or the resin depending on the type of detergent. The freed protein binds to the resin and the detergent moves along the liquid phase and is discarded with the flowthrough. The bound protein is then eluted in a detergent-free buffer.

**What proteins can be processed using this kit?** All soluble (acidic and basic) proteins can be processed using this kit.

**What can I do if I am not aware of the isoelectric point (pI) of my protein of interest?** The isoelectric point of a protein (pI) may be theoretically computed from its amino acid composition through the aid of web-based applications such as [http://us.expasy.org/tools/pi\\_tool.html](http://us.expasy.org/tools/pi_tool.html). Another site at [http://www.up.univ-mex.fr/~mahim/d\\_abim/compo-p.html](http://www.up.univ-mex.fr/~mahim/d_abim/compo-p.html) contains considerations for the position of residues in the polypeptide chain to determine isoelectric points. The calculated pI should help determine whether the acidic or basic protocol is to be followed. Alternatively, if there is sufficient protein sample, the protein can be bound and eluted using first the acidic protocol, and then the basic protocol, and the recovery compared to determine which protocol gives better results.

**Can the columns be reused?** Columns are designed for single use only. This minimizes sample carryover.

## Frequently Asked Questions and Troubleshooting

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**How should I store the Detergent Clean-Up Micro Kit 25 solutions?** Once opened, the solution containers should be stored at 4 °C when not in use. If any precipitation of the reagents occurs, warm to room temperature and dissolve the crystals prior to use. If unopened, the solution containers can be stored at room temperature.

**Do I need to pre-filter my sample before loading it onto the column?** Insoluble materials, if present in the protein solution, should be spun down and the clarified solution applied to the column.

**How should I prepare my sample before loading it onto the column?** Ensure that the pH of the sample is the same as the pH of the Column Activation and Wash Solution that you are using. Ensure that you have added one volume of the Emulsifier to your sample. Also, ensure that the amount of protein in your sample will not overload the column capacity (50 µg).

**What are the maximum and minimum sample volumes that I can load onto the column?** We recommend that no more than 1 mL of sample be loaded onto the column, to ensure that the tip of the column does not rest in the flowthrough that is caught in the collection tube. The minimum load for binding must be at least 100 µL to completely cover the resin bed.

**What are the maximum and minimum amounts of protein that I can load onto the column?** We recommend that between 2 µg - 50 µg of sample be loaded onto the column in order to obtain high recovery.

**What are the highest and lowest recommended microcentrifuge speeds for the columns?** We recommend a speed between 10,000 and 14,000 rpm in microcentrifuges (maximum 15,000 × g). Speeds below 10,000 rpm may be insufficient to completely move the liquid phase through the resin bed. Additional spinning times may be required to remove this liquid.

## Frequently Asked Questions and Troubleshooting

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**What is the typical amount of protein recovered that I can expect with the ProteoSpin™ columns?**

**Recovery of BSA in water was found to be greater than 90% when testing 50 µg of protein. Recovery depends on the specific protein sample, and varies with detergent type and concentration.**

**Can I autoclave the columns?** No. The columns are not designed to be autoclaved as it causes the plastic in the columns to deform.

**Can all types of detergents be removed using this kit? What about SDS?** Yes, the kit is capable of removing all types of detergents including anionic, cationic, zwitterionic and non-ionic. SDS can be removed efficiently with the kit, including tightly bound SDS complexes.

**How can I tell if my protein bound correctly to the column?** Save the flowthrough from the binding step and perform a protein assay to determine the amount of protein present.

**I used the recommended binding pH, but still my protein did not bind correctly. Can**

**I use a different binding pH?** Yes. The binding pH may be adjusted depending on the *pI* of your protein. It is suggested to start with a pH that is one unit lower than the *pI*, and if necessary, lowering the pH from there.

**I use a colorimetric protein assay to determine concentration of eluted proteins. Is there an accurate way of doing it?** The colorimetric protein assays available in the market are quite accurate, however, there are a number of ways to improve them. First, using a linear regression to fit the points for the standard curve is often less accurate than using a second-degree polynomial. Microsoft® Excel has full features for curve fitting. The goodness-of-fit for the regression should determine whether first-degree (linear) or second-degree (polynomial) would be used. Second, the protein used as the standard should closely match the dye-binding characteristics of the protein of interest. For example, using BSA as a standard to determine concentrations of immunoglobulins may provide erroneous results. This is because immunoglobulins do not bind some dyes efficiently. Third, ensure that the protein standard is in a solution that resembles the solution of the test protein. Sometimes buffer constituents can have drastic effects on dye binding properties.

## Frequently Asked Questions and Troubleshooting

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**The acidic and basic methods are almost identical, except for the solutions used. Why is it necessary to separate the two?** In ion exchange chromatography, it is customary to use either a cation or anion exchanger resin, depending on the type of target protein. For SFC resin, which carries negative charges on its surface, proteins bind through positive charges on their side chains that are exposed on the surface. In theory, the acidic method should suffice to capture all basic and acidic proteins. In practice, however, we find that basic proteins bound under acidic conditions are difficult to elute, and therefore decrease the overall performance of the columns. Binding basic proteins at pH 7 reduces column affinity for such proteins, making elution more efficient.

**The binding pH is 4.5, yet my protein has a pI of 4. Do I need to adjust the pH of my binding solution to lower than 4.5? If so, how would I do that?** If your protein has a pI lower than 4.5, it is almost always necessary to lower the pH of the protein solution so that it is one pH unit lower than the protein's pI. If the ProteoSpin pH Binding Buffer for Acidic Proteins is used, the final pH may be adjusted to as low as 3.75 by titrating with HCl. If a lower binding pH is desired, you may use different buffers, such as phosphate ( $pK_{a1} = 2.15$ ). Many acidic proteins tend to precipitate as the pH of the solution approaches their pI. If protein precipitation is apparent (solution becomes cloudy), diluting the solution to a lower protein concentration may solve the problem. Using a binding pH of one-half unit below the protein's pI is also feasible, although the expected recovery for the bound protein will be lower.

## Frequently Asked Questions and Troubleshooting

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### Troubleshooting Guide

Each of the following tables discusses problems that may occur, their possible causes, and presents solutions and explanations.

#### Problem: Protein Solution Does Not Flow Through the Column

Possible Causes	Solution & Explanation
Centrifugation speed was too low.	Check the centrifuge to ensure that it is capable of generating 14,000 $\times g$ . Sufficient centrifugal force is required to move the liquid phase through the resin.
Inadequate spin time.	Spin an additional minute to ensure that the liquid is able to flow completely through the column.
Protein solution is too viscous.	Dilute the protein solution and adjust the pH accordingly. Highly viscous materials due to high protein concentration can retard the flow rate.
Cellular debris is present in protein solution.	Filter the sample in a 0.45 $\mu\text{m}$ filter or spin down insoluble materials and transfer the liquid portion to the column. Solid, insoluble materials can cause severe clogging problems.
Protein solution is not completely dissolved.	Dissolve the sample in a larger volume of buffer. Solid, insoluble materials can cause severe clogging problems.

## Frequently Asked Questions and Troubleshooting

### Problem: Poor Protein Recovery

Possible Causes	Solution & Explanation
Incorrect procedure was used.	Ensure that the acidic protocol was used for an acidic protein, and the basic protocol for a basic protein. It is known that when basic proteins are bound using acidic protocol, elution is inefficient because the basic proteins are bound tightly.
Initial volume of sample applied to column was too low.	Load at least 100 $\mu\text{L}$ onto the column. This volume is required to cover the resin bed completely.
Incorrect pH adjustment of sample.	Ensure that the pH of the sample is 4.5 for acidic proteins and 7.0 for basic proteins.
Emulsifier was not added.	Ensure that one volume of Emulsifier was added to the sample and the pH re-verified prior to loading onto the column.
Protein may have precipitated prior to loading into the column.	If the pH of the protein solution is the same as the pI of your protein, precipitation may occur. In this case, adjust the pH of your sample to at least one pH unit lower than the pI of your protein.

### Problem: Eluted Protein Is Degraded

Possible Causes	Solution & Explanation
Eluted protein solution was not neutralized.	Add 5 $\mu\text{L}$ of Neutralizer to each 50 $\mu\text{L}$ of eluted protein in order to adjust the pH to neutral. Some proteins are sensitive to a high pH, such as the elution buffer at pH 12.5.
Eluted protein solution was not neutralized quickly enough.	If the eluted protein is not used immediately, degradation will occur. We strongly suggest adding Neutralizer in order to lower the pH.

## Frequently Asked Questions and Troubleshooting

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### Problem: Eluted Protein Is Degraded

Possible Causes	Solution & Explanation
Proteases may be present.	Use protease inhibitors during all steps of sample preparation.
Bacterial contamination of the protein solution.	Prepare the protein samples with 0.015% sodium azide. The Elution Buffer already contains sodium azide.



## Related Products

# D

The following products are available from MDS Sciex.

### CBED - Related Products

Product Name	Part Number	# Samples	mg SiC	Protein Capacity
ProteoSpin CBED Micro Kit 25	1006089	25	25	50 µg
ProteoSpin CBED Micro Kit 50	1006360	50	25	50 µg
ProteoSpin CBED Micro Kit 250	1006361	250	25	50 µg
ProteoSpin Inclusion Body Isolation Micro Kit 20	1006092	20	25	50 µg
ProteoSpin Inclusion Body Isolation Micro Kit 50	1006366	50	25	50 µg
SiC Micro Spin Columns/2 mL Collection Tubes, 250	1006367	250	25	50 µg
Collection Tubes, 2 mL, 250	1006368	250		
Final Collection Tubes, 1.7 mL, 300	1011298	300		

Related Products

ProteoLadder Protein Markers	Part Number	# Samples	High Intensity Band(s)
ProteoLadder 100, 100 Lanes	1006355	100	50 kDa
ProteoLadder 125, 100 Lanes	1006356	100	60 kDa
ProteoLadder 150, 100 Lanes	1006354	100	50, 100 kDa
ProteoLadder 100, 10 Lanes Lyophilized	1011502	10	50 kDa
ProteoLadder 100, 100 Lanes Lyophilized	1011499	100	50 kDa
ProteoLadder 125, 10 Lanes Lyophilized	1011503	10	60 kDa
ProteoLadder 125, 100 Lanes Lyophilized	1011500	100	60 kDa
ProteoLadder 150, 10 Lanes Lyophilized	1011504	10	50, 100 kDa
ProteoLadder 150, 100 Lanes Lyophilized	1011501	100	50, 100 kDa

## Related Products

MDS Sciex dsDNA Markers 1 mL volume	Product Number	High Intensity Band(s), bp	# Bands	Application
dsDNA Marker 25-650 bp 100 Lanes	1006370	200, 400	14	PCR size confirmation
dsDNA Marker 50-1,000 bp 100 Lanes	1006345	500	11	PCR products
dsDNA Marker 100-1,000 bp 100 Lanes	1006346	500	10	PCR products
dsDNA Marker 100-2,000 bp 100 Lanes	1006347	500	11	Fast run times
dsDNA Marker 100-2,686 bp 100 Lanes	1006348	500	14	DNA clones
dsDNA Marker 300-5,000 bp 100 Lanes	1006349	None	10	Larger PCR products, cloning
dsDNA Marker 100-5,000 bp 100 Lanes	1006350	500, 1000	16	Larges, small cloning
dsDNA Marker 300-10,000 bp 100 Lanes	1006351	None	13	Digested DNA
dsDNA Marker 100-10,000 bp 100 Lanes	1006352	500, 1000, 5000	19	Digested DNA
dsDNA Marker 300-24,000 bp 100 Lanes	1006353	5000, 10000	15	High MW digested DNA

Related Products

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# ProteoSpin™

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## A Technology Partnership

MDS Sciecx's partners enhance our expertise in the life sciences market. Norgen Biotech joins MDS Sciecx to provide you with the most comprehensive and cost-effective ProteoSpin™ protein purification products, ProteoLadder™ Protein Markers and DNA Markers. ProteoSpin™ products are manufactured by Norgen Biotech Corp.

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