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ZYMO RESEARCH
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INSTRUCTION MANUAL

Strep-Spin Protein Miniprep Kit™

Catalog No. **P2004 & P2005**

Highlights

- **Fast & Simple:** Purify Strep-tagged proteins from cell-free extracts using a spin-column in ≥7 minutes
- **High-Quality:** Easy way to prepare pure protein for small-scale studies
- **Convenient:** No special instrumentation needed other than a benchtop microcentrifuge

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ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

Product Contents

Strep-Spin Protein Miniprep™ (Kit Size)	P2004 (10 Preps.)	P2005 (50 Preps.)	Storage Temperature
Zymo-Spin™ P1 columns	10	50	Room Temp.
Collection Tubes	10	50	Room Temp.
Strep-Tactin® XT Superflow® 50% suspension[1]	1 ml	5 ml	4°C
Strep-Wash Buffer[1]	15 ml	65 ml	4°C
Strep-Elution Buffer[1]	6 ml	30 ml	4°C
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Note - Integrity of kit components is guaranteed for 6 months from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

All components available for purchase separately. For ordering information, refer to page 8.

[1] The buffers should be stored refrigerated. However, they may be stored for up to 3 months 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 Strep-Tactin® XT Superflow® 50% suspension may be kept at room temperature for up to 7 days.

Specifications:

- **Processing Time:** 7 minutes
- **Protein Purity:** Electrophoretically pure. Purified high-quality protein is suitable for enzyme kinetics, protein biochemical analyses, SDS-PAGE, and other applications.
- **Elution Volume:** Recommended 3 x 200 µl
- **Elution Method:** Biotin excess
- **Affinity Matrix:** Strep-Tactin® XT Superflow® 50%
- **Capacity:** 100 µl of Strep-Tactin® XT Superflow® 50% suspension can bind 15 nmol biotin (approx. 450 µg of a 30 kDa Twin-Strep-tag® protein).
- **Required Equipment:** Microcentrifuge
- **Principle of Technology:** The Strep-Spin Protein Miniprep Kit is based on a novel Strep-Tactin® XT Superflow® resin which binds to Twin-Strep-tag® with very high affinity. This tag contains two Strep-tag® II peptides connected via a Glycine/Serine linker sequence. Hence this kit is optimized for the purification of Twin-Step®-tagged proteins; however it will also work efficiently for proteins with single Strep-tags.

○ Strep-tag® II amino sequence: WSHPQFEK

○ Twin-Strep-Tag® (Strep-tag III) amino acid sequence: WSHPQFEKGGGSGGGSGGWSHPQFE

Notes:

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Description

The **Strep-Spin Protein Miniprep Kit™** provides a fast purification technology for Strep-tagged proteins. Up to 450 µg of Strep-tagged protein can be purified in 7 minutes and eluted in **Strep-Elution Buffer**. The purified protein can be used directly for enzymatic assays, protein biochemical analyses, SDS-PAGE and other applications. The product has been optimized for maximal protein purity (Figure 1). The straightforward spin – wash – elute protocol dramatically simplifies protein purification: get results in minutes, not hours.

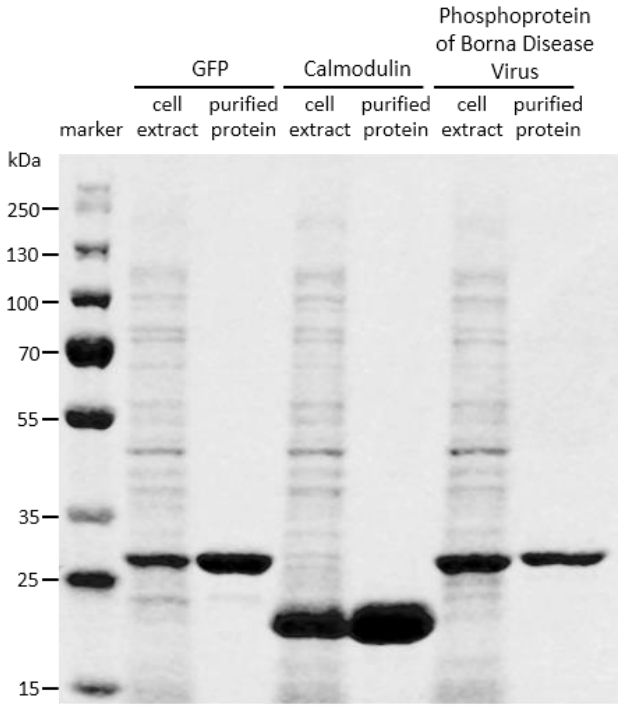

Figure 1. *E. coli* cell extracts containing indicated proteins expressed as a C-terminal Twin-Strep-tag fusion, as well as the proteins purified using Strep-Spin Protein Miniprep™ were analyzed by SDS-PAGE on a 15% gel, and stained with Coomassie Blue®. (GFP 28 kD, Calmodulin 19,8 kD, BDV-P 25,5 kD)

For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Procedure Overview:

Sample Preparation

Any cell extract or other complex protein mixtures containing Strep-tagged protein can be used as a starting material as long as the proteins are soluble. The pH value of the loaded sample should be >7.0. The sample should not contain any biotin, as this will prevent the protein from binding to the matrix. Sample may contain up to:

Reduction Agents	Concentration
β-mercaptoethanol	≤ 50 mM

Non-Ionic Detergents	Concentrations
Nonidet® P-40	≤ 2%
Triton® X-100	≤ 2%
Tween®-20	≤ 2%

Ionic Detergents	Concentration
SDS; Sodium-N-dodecyl sulfate	≤ 0.1%

Denaturing Reagents	Concentrations
Urea	≤ 8 M

Chemicals and other Reagents	Concentrations
EDTA	≤ 50 mM
Ethanol	≤ 10% (v/v)
Glycerol	≤ 25% (v/v)
Imidazole	≤ 250 mM
$MgCl_2$	≤ 1 M
NaCl	≤ 5 M

Protocol:

The procedure can be conducted in the cold or at room temperature. We recommend using cold buffers and working on ice for sensitive proteins. Please pay careful attention to centrifugation times: the times listed include the time needed for acceleration. Centrifugation steps are carried out in a standard tabletop microcentrifuge at **maximum speed**, which usually corresponds to **13,000-15,000 *x g***.

1. Transfer 100 µl of **Strep-Tactin® XT Superflow® 50%**[1] to the **Zymo-Spin™ P1 Column** and place the column into a **Collection tube**.

   *Use a 1 ml pipette tip to transfer the **Strep-Tactin® XT Superflow® 50%**; 200 µl-size or smaller automatic pipette tips have a small opening and may not be large enough for the resin particles.*

2. Centrifuge for 10 seconds[2].
3. Load 200 µl **Strep-Wash Buffer** to equilibrate the resin.
4. Centrifuge for 10 seconds.
5. Add ≤ 800 µl protein sample and resuspend the resin by tapping or inverting the column. Incubate for 1 minute[3].
6. Centrifuge the column/collection tube assembly for 20 seconds. Discard the flow-through and place the column back into the Collection tube.
7. Add 500 µl of **Strep-Wash Buffer** and resuspend the resin. Centrifuge for 20 seconds. Discard the flow-through.
8. Add 200 µl of **Strep-Wash Buffer** and resuspend the resin. Centrifuge for 20 seconds. Discard the flow-through.
9. Repeat **Step 8** two more times.
10. Place the **Zymo-Spin™ P1 Column** into a standard microcentrifuge tube. Add 200 µl[4] of **Strep-Elution Buffer** to the column, resuspend the resin, and incubate for 1 minute.
11. Centrifuge for 20 seconds to elute the purified protein.
12. Repeat **Steps 10 and 11** two more times in new microcentrifuge tubes to obtain three elution fractions in total[5].

The eluates contain the purified protein and can be combined. The eluted protein is suitable for many applications. Use 1-10 µl for SDS-PAGE and Coomassie blue staining analysis. Store the purified protein at appropriate temperature.

Notes:

[1] Completely resuspend the Strep-Tactin® XT Superflow® 50% by mixing or vortexing immediately before use.

[2] Ensure that the Strep-Tactin® XT Superflow® 50% is completely drained. Some older centrifuge models may require longer time of centrifugation.

[3] If sample volume is > 800 µl, column can be reloaded.

[4] Smaller elution volumes are possible and may yield more concentrated protein, but the elution efficiency may be compromised.

[5] For higher total yields, more elution steps may be performed.

Appendix A: Buffer Composition

Strep-Wash Buffer	
100 mM	Tris/HCl
150 mM	NaCl
1 mM	EDTA
pH	8.0

Strep-Elution Buffer	
100 mM	Tris/HCl
150 mM	NaCl
1 mM	EDTA
50 mM	Biotin
pH	8.0

Appendix B: Suggested preparation of cleared lysate

For protein purification from *E. coli* lysates:

1. Harvest & pellet 10 ml of E. coli culture.
2. Resuspend in 1-2 ml of Lysis Buffer
   (e.g. 300 mM NaCl,1% NP-40 in PBS, 1 mg/ml Lysozyme, 1 x Protease Inhibitor, 15 U/ml Benzonase®).
3. Incubate for 30 minutes at room temperature.
4. Spin at ≥ 12,000 x g at 4°C for 5 minutes.
5. Use the supernatant for the Strep-Spin Protein Miniprep Kit™ protocol.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Protein recovery	
Protein not eluted	▪ **Starting material contains Biotin.** Free biotin binds to the Strep-Tactin® XT matrix and thus reduces the binding capacity for the desired protein. To remove biotin from the sample we recommend to add stoichiometric amounts of Avidin prior to using the kit. Avidin blocks the biotin but does not bind to the Strep-tag. Alternatively, dialysis could be conducted ▪ **Protein folding.** There are several possible explanations for recovering no protein. The Strep-tag may be rendered inaccessible due to protein folding. ▪ **Protein insoluble**. The recombinant protein can also be insoluble as a result of overexpression. In both cases, the protein can be purified at denaturing conditions (see below) ▪ **Too much detergent used.** Please ensure not to use higher detergents volumes as mentioned in the Sample Preparation table. Using higher detergent amounts can reduce binding affinity.
Insoluble protein	▪ **Optimize expression conditions.** Overexpression of proteins may result in formation of insoluble inclusion bodies inside cells. If a large band of over-expressed protein is visible after SDS-PAGE electrophoresis of whole cells, but the band is absent after SDS-PAGE electrophoresis of cleared cell lysates, this indicates that the protein may not be soluble and the expressed protein may form inclusion bodies. ▪ **Use Denaturing conditions.** Insoluble proteins will not be purified using the provided buffers. It is, however, possible to purify such proteins at denaturing conditions in the presence of ≤ 8 M urea or ≤ 6 M guanidine hydrochloride. The protein native structure and thus enzyme activity is lost under such conditions, but may be restored by refolding the protein after purification.
Diluted starting material	▪ **Reload Column.** If the starting material contains only low levels of Strep-tagged protein and requires more than 800 µl sample volume to purify enough protein, the spin column can be reloaded (repeat Steps 5 and 6 of the Protocol)
Membrane associated protein	▪ **Use Detergent.** Membrane proteins can be purified after solubilization in a nonionic detergent. Concentrations of up to 2% of Triton® or TWEEN® can be present in the loaded sample
Protein Purity	
Eluted protein is not pure	▪ **Buffer contamination.** Check your buffers for signs of contamination, and check the pH of the buffers. ▪ **Increase centrifugation time and speed.** Make sure that centrifugation drains the Strep-Tactin® XT Superflow® completely after each spin (some older centrifuge models may require longer centrifugation time). ▪ **Additional Washing.** If the problem persists, add additional wash steps in the purification protocol.

Ordering Information

Product Description	Kit Size	Catalog No.
Strep-Spin Protein Miniprep™	10 preps.	P2004
Strep-Spin Protein Miniprep™	50 preps.	P2005

For Individual Sale	Size	Catalog No
Zymo-Spin™ P1 Columns	50	P2003-1
Collection Tubes	50	C1001-50
Strep-Tactin® XT Superflow® 50%	5 ml	P2004-1-5
Strep-Wash Buffer	65 ml	P2004-2-65
Strep-Elution Buffer	30 ml	P2004-3-30

Other Protein Purification Kits

Product Description	Kit Size	Catalog No.
His-Spin Protein Miniprep™	10 preps.	P2001
His-Spin Protein Miniprep™	50 preps.	P2002
MBP-Spin Protein Miniprep™	10 preps.	P2006
MBP-Spin Protein Miniprep™	50 preps.	P2007

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