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INSTRUCTION MANUAL

MBP-Spin Protein Miniprep Kit™

Catalog Nos. **P2006 & P2007**

Highlights

- **Fast & Simple:** Purify MBP-tagged proteins from cell-free extracts using a spin-column in ≥6 minutes.
- **High-Quality:** Prepare pure proteins for small-scale studies using a spin-column.
- **Convenient:** No special instrumentation needed other than a benchtop microcentrifuge.

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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:

MBP-Spin Protein Miniprep™ (Kit Size)	P2006 (10 Preps.)	P2007 (50 Preps.)	Storage Temperature
Zymo-Spin™ P1 columns	10	50	Room Temp.
Collection Tubes	10	50	Room Temp.
Amylose Resin	1 ml	5 ml	4°C
MBP-Wash Buffer[1]	14 ml	70 ml	Room Temp.
MBP-Elution Buffer[1]	4 ml	20 ml	Room Temp.
Instruction Manual	1	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

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

[1] The MBP-Wash Buffer and MBP-Elution Buffer should be kept cold or put on ice prior to use for purification of sensitive proteins.

Specifications:

- **Sample Type:** Cell lysates or other complex protein mixtures containing MBP-tagged proteins.
- **Processing Time:** 6 minutes
- **Protein Purity:** Electrophoretically pure and suitable for enzyme kinetics, biochemical analyses, SDS-PAGE, and other applications.
- **Elution Volume:** ≥ 200 µl
- **Elution Method:** Excess Maltose
- **Affinity Matrix:** Amylose Resin
- **Capacity:** 100 µl of Amylose Resin typically binds ≥400 µg of fusion protein.
- **Required Equipment:** Microcentrifuge
- **Principle of Technology:** The MBP-Spin Protein Miniprep Kit uses an affinity matrix composed of amylose resin to specifically bind proteins fused to maltose-binding protein (MBP). MBP is known to significantly enhance the solubility of many proteins when fused to them. We recommend using NEB pMAL-c5X or pMAL-p5X for the construction of the fusion plasmid.

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 **MBP-Spin Protein Miniprep Kit™** provides a fast purification technology for MBP-tagged proteins. Up to 1000 µg of MBP-tagged protein can be purified in 6 minutes and eluted in **MBP-Elution Buffer**. The purified protein is ultra-pure (Figure 1) and can be used directly for enzymatic assays, biochemical analyses, SDS-PAGE and other sensitive applications. The straightforward spin, wash, and elute protocol dramatically simplifies protein purification and allows the end user to get results in minutes, not hours.

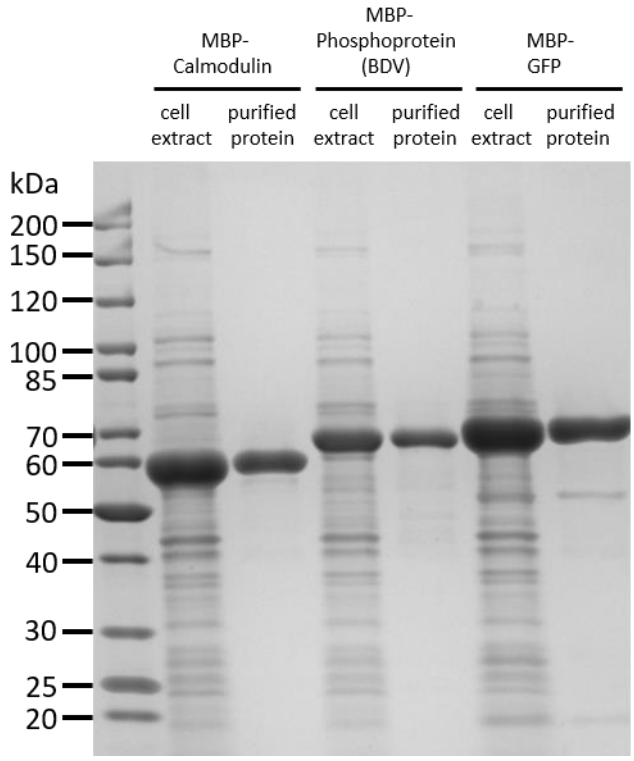

Figure 1. The MBP-Spin™ Protein Miniprep Kit purifies high-quality MBP-tagged proteins directly from a spin-column. N-terminal MBP-tag fusion proteins were expressed in E. coli cells, and the cell extracts as well as the proteins purified using the MBP-Spin™ Protein Miniprep Kit were analyzed by SDSPAGE on a 4-20% gel and stained with InstantBlue™; (MBP-Calmodulin 55 kDa, MBP-BDV-Phosphoprotein 65 kDa, MBP-GFP 69 kDa).

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 MBP-tagged protein can be used as a starting material as long as the proteins are soluble. For most proteins, the ideal pH for sample input is 7.0, however this may vary from protein to protein. The sample should not contain any maltose, as this will prevent the protein from binding to the matrix. The following concentrations of commonly used chemicals have been tested with several different proteins without effecting protein purification:

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%

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

Protocol:

The following procedure can be performed cold (4–8°C) or at room temperature (15–30 °C). Centrifugation steps are carried out in a standard tabletop microcentrifuge at maximum speed, which usually corresponds to 13,000-17,000 *x g*.

1. Place the **Zymo-Spin™ P1 Column** in a collection tube.
2. Completely resuspend the **Amylose Resin** by vortexing or pipetting and transfer 100 µl of Amylose Resin to the Zymo-Spin™ P1 Column.

   Use a 1 ml micropipette tip to transfer the Amylose Resin. 200 µl micropipette tips or smaller have tiny openings and may not be large enough for the resin particles. For easier pipetting, the very tip of the micropipette tip can be cut off.
3. Centrifuge the Zymo-Spin™ P1 Column for 15 seconds[1].
4. Load 500 µl of **MBP-Wash Buffer** to equilibrate the resin.
5. Centrifuge the Zymo-Spin™ P1 Column for 15 seconds. Discard the flow-through.
6. Add ≤ 800 µl of protein sample to the Zymo-Spin™ P1 Column and resuspend the resin by pipetting or tapping the column. Incubate the column for 3 minutes and resuspend the resin several more times during the incubation[2].
7. Centrifuge the Zymo-Spin™ P1 Column for 15 seconds. Discard the flow through and place the column back into the collection tube[3].
8. Add 500 µl of MBP-Wash Buffer to the Zymo-Spin™ P1 Column and resuspend the resin. Centrifuge for 15 seconds and discard the flow-through.
9. Add 200 µl of MBP-Wash Buffer to the Zymo-Spin™ P1 Column and resuspend the resin. Centrifuge for 15 seconds.
10. Repeat step 9.
11. Transfer the Zymo-Spin™ P1 Column into a clean 1.5 ml microcentrifuge tube. Add 200 ul of **MBP-Elution Buffer** to the column[4], resuspend the resin and incubate for 1 minute. Centrifuge for 15 seconds to elute the purified protein.

The eluate contains the purified protein, which is suitable for many applications. Use 1-10 µl for SDS-PAGE and Coomassie blue staining analysis. Store the purified protein at the appropriate temperature.

Notes:

[1] Ensure that the Amylose resin is completely drained. Some older centrifuges may require a longer centrifugation time.

[2] Longer incubation times can increase yield.

[3] For sample volumes larger than 800 µl, repeat steps 6 and 7 until the entire sample has been loaded onto the resin.

[4] A lower elution volume may be used if more concentrated protein is desired. For higher total yield, multiple elutions may be performed.

Appendix A: Buffer Composition

MBP-Wash Buffer	
20 mM	Tris/HCl
200 mM	NaCl
1 mM	EDTA
pH	7.4

MBP-Elution Buffer	
20 mM	Tris/HCl
200 mM	NaCl
1 mM	EDTA
10 mM	Maltose
pH	7.4

Appendix B: Suggested Preparation of Cleared Lysate

Protein purification from E. coli lysates:

1. Harvest 10 ml of culture grown in LB supplemented with 0.2 % glucose.
2. Resuspend the cell pellet in ≥ 2 ml of Lysis Buffer
   (e.g. 300 mM NaCl, 1 % Nonidet® P-40 in PBS, 1 mg/ml Lysozyme, 1x Protease Inhibitor, 15 U/ml Benzonase).
3. Incubate for 30 minutes at room temperature.
4. Sonicate 3 times for 30 seconds with 30 second incubations on ice in between each sonication.
5. Spin at ≥ 12,000 *x g* at 4°C for 5 minutes.
6. Use 800 µl of the recovered supernatant for the MBP-Spin Protein Miniprep protocol.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low Protein Yield	
Protein does not bind	• **Starting material contains Maltose.** Free maltose binds to the Amylose Resin and thus reduces the binding capacity for the desired protein. • **Too much detergent used.** Nonionic detergents or other chemicals present in the crude extract can reduce binding affinity. Some fusion proteins bind less efficiently in the present of detergents than others. A reduction in the detergent concentration or using another lysis buffer may help. • **Growth medium used for protein expression.** Cells grown in LB and similar media have substantial amounts of an amylase that interferes with binding. Expression of this amylase is repressed by including glucose (0.2 %) in the media.
Insoluble protein	▪ **Optimize expression conditions.** Overexpression of proteins may result in the formation of insoluble inclusion bodies inside cells. If a large band of over-expressed protein is visible after SDS-PAGE electrophoresis of intact cells, but not present in cleared cell lysates, then the expressed protein may not be soluble or form inclusion bodies. Try expressing the protein at lower temperatures.
Starting material too dilute	• **Reload Column.** If the starting material contains very low levels of MBP-tagged protein, then it may require more than 800 µl of sample volume to purify enough protein. Simply repeat steps 6 and 7 until the desired volume has been loaded onto the column.
Poor Protein Quality	
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 Amylose Resin 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.
MBP-Spin Protein Miniprep™	10 preps.	P2006
MBP-Spin Protein Miniprep™	50 preps.	P2007

For Individual Sale	Size	Size
Zymo-Spin™ P1 Columns	50	P2003-1
Collection Tubes	50	C1001-50
Amylose Resin	5 ml	P2006-1-5
MBP-Wash Buffer	70 ml	P2006-2-70
MBP-Elution Buffer	20 ml	P2006-3-20

Other Protein Purification Kits

Product Description	Kit Size	Catalog No.
His-Spin Protein Miniprep™	10 preps.	P2001
His-Spin Protein Miniprep™	50 preps.	P2002
Strep-Spin Protein Miniprep™	10 preps.	P2004
Strep-Spin Protein Miniprep™	50 preps.	P2005

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