INSTRUCTIONS

Pierce Anti-c-Myc Agarose

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20168 20169

Number	Description
20168	**Pierce Anti-c-Myc Agarose**, 2mL settled resin Support: Superflow 6 resin, highly crosslinked 6% agarose supplied as 25% slurry (e.g., 1mL of settled resin is equivalent to 4mL of 25% slurry) Supplied: 1:3 suspension in 0.1M phosphate, 0.15M NaCl, pH 7.2 with 0.05% sodium azide Loading: 3.5mg mouse anti-c-Myc IgG_1 monoclonal antibody conjugated per mL of settled agarose resin
20169	**Pierce Anti-c-Myc Agarose**, 10mL settled resin Support: Superflow 6 resin, highly crosslinked 6% agarose supplied as 25% slurry (e.g., 1mL of settled resin is equivalent to 4mL of 25% slurry) Supplied: 1:3 suspension in 0.1M phosphate, 0.15M NaCl, pH 7.2 with 0.05% sodium azide Loading: 3.5mg mouse anti-c-Myc IgG_1 monoclonal antibody conjugated per mL of settled agarose resin

Storage: Upon receipt store at 4°C. Do not freeze. Product is shipped with an ice pack.

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Introduction

The Thermo Scientific™ Pierce™ Anti-c-Myc Agarose is an immunopurification and immunoprecipitation resin specific for c-Myc-tagged proteins expressed in human *in vitro* expression systems and bacterial and mammalian cell lysates. The anti-c-Myc antibody coupled to the resin is a high-affinity mouse IgG_1 monoclonal antibody that recognizes the c-Myc-epitope tag (EQKLISEEDL) derived from the C-terminus of human c-Myc protein. Pierce Anti-c-Myc Agarose can be used in gravity purification columns, spin purification columns or cartridges for FPLC instruments.

Important Product Information

- For best results, determine optimal conditions for expression of c-Myc-tagged fusion protein before attempting immunoprecipitation or immunopurification.
- For optimal results, add protease inhibitors (e.g., Thermo Scientific™ Halt™ Protease Inhibitor Cocktail, Product No. 87786) when preparing any lysate.

- Binding capacity of c-Myc-tagged fusion protein is 102-144nmol per mL of settled resin. Elution capacity of the c-Myc-tagged fusion protein is minimally 18-19nmol per mL of settled resin when using 0.1M glycine, pH 2.8. Binding and elution capacity will vary depending on the c-Myc-fusion protein and the method of elution. Binding and elution capacities are based on a 26kDa c-Myc-tagged protein.
- Thoroughly resuspend the Pierce Anti-c-Myc Agarose by inverting the bottle several times before dispensing. Do not vortex.

Additional Materials Recommended

- Halt Protease Inhibitor Cocktail (Product No. 87786)
- Tween™-20 Detergent (e.g., Thermo Scientific™ Surfact-Amps™ 20 Detergent Solution, Product No. 28320)
- End-over-end rocker or rotator
- 1M Tris, pH 9.5
- 0.1M Glycine, pH 2.0-2.8
- 3M NaSCN
- 50mM NaOH
- Thermo Scientific™ Pierce™ c-Myc Peptide (Product No. 26184)
- Reagent for lysing cells, such as Thermo Scientific™ M-PER™ Mammalian Protein Extraction Reagent (Product No. 78501) or Thermo Scientific™ B-PER™ Bacterial Protein Extraction Reagent (Product No. 78243)
- Spin columns and collection tubes (e.g., Thermo Scientific™ Pierce™ Spin Columns, 0.9mL, Product No. 69705 or Pierce Centrifuge Columns, 5mL, Product No. 89897)
- Tris-buffered saline (TBS) (e.g., Thermo Scientific™ BupH™ Tris Buffered Saline Packs, Product No. 28376)

Procedure for Lysis of Mammalian Cells

Note: For optimal results, use a protease inhibitor cocktail, such as Halt Protease Inhibitor Cocktail (Product No. 87786), when preparing any cell lysate

A. Lysis of Adherent Mammalian Cells

1. Carefully decant culture medium and rinse the cells once with ice-cold TBS.
2. Add the volume of M-PER Reagent to the plate or well as indicated in Table 1. Gently shake plate for 5 minutes.
3. Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at 16,000 × *g* at 4ºC for 20 minutes to pellet the cell debris.

Table 1. Recommended volumes of Thermo Scientific M-PER Reagent.

Plate Type	Volume of M-PER Reagent
100mm (diameter)	500-1000µL
60mm (diameter)	250-500µL
6-well plate	200-400µL/well
24-well plate	100-200µL/well

B. Lysis of Non-adherent Mammalian Cells

1. Centrifuge the cell suspension at 500 × *g* for five minutes to pellet the cells. Discard the supernatant.
2. Wash cells once by resuspending the cell pellet in ice-cold TBS. Centrifuge at 500 × *g* for five minutes to pellet cells.
3. Add M-PER Reagent to the cell pellet (500µL of M-PER Reagent is sufficient for lysing 50mg of wet cell pellet). For optimal results use a 10:1 v/w ratio.
4. Gently shake the sample for 10 minutes. Remove cell debris by centrifugation at 16,000 × *g* at 4ºC for 20 minutes.

Procedure for Lysis of Bacterial Cells

1. Pellet bacterial cells by centrifugation at 5000 × *g* for 10 minutes.
2. Optional: Add 2µL of lysozyme and 2µL of DNAse I per 1mL of B-PER Reagent. Add protease inhibitors.
3. Add 4mL of B-PER Reagent per gram of cell pellet. Pipette the suspension up and down until it is homogeneous.

   Note: If using B-PER II Reagent, 2mL of reagent per gram of cell pellet may be used to achieve a more concentrated protein solution.
4. Incubate 10-15 minutes at room temperature.
5. Centrifuge lysate at 15,000 × *g* for five minutes to separate soluble proteins from insoluble proteins.

Procedure for IP of c-Myc-tagged Protein

Note: The amount of lysate needed and incubation time are dependent upon the expression level, type of c-Myc-tagged protein, and type of lysate. Optimization may be required for each specific system.

A. Immunoprecipitation Using Spin Columns or Microcentrifuge Tubes

1. Add 20-100µL of Pierce Anti-c-Myc Agarose slurry to tube. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Discard liquid.
2. Wash resin with one resin volume of TBS. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Discard liquid.
3. Add lysate to tube. Bring total volume of lysate to at least 200µL with TBS. For c-Myc-tagged proteins produced using the Thermo Scientific™ Pierce™ *In Vitro* Protein Expression Kits, dilute lysate for a final volume of 200µL in TBS.
4. Incubate one hour to overnight at 4ºC with gentle end-over-end mixing or a rocking platform.
5. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Save the supernatant for analysis of binding efficiency.
6. Prepare a wash solution of TBS with 0.05% Tween-20 Detergent (TBS-T).
7. Wash resin with 500µL of TBS-T and invert the column several times. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Discard wash. Repeat this step two additional times.

B. Elution of c-Myc-tagged Protein

Note: Select one of the elution protocols below. If the eluted c-Myc-tagged protein will be used for function applications or is sensitive to pH extremes or sodium thiocyanate, then elute the protein with Pierce c-Myc Peptide.

- **Gentle Elution Protocol:**

1. Prepare Pierce c-Myc Peptide at 0.5 mg/mL in TBS.
2. Add one bed volume of 0.5mg/mL Pierce c-Myc Peptide and incubate for 10-15 minutes at 37°C. Elution may be performed at reduced temperatures; however, lower yields may result.
3. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Collect eluate.
4. Repeat Steps 2 and 3 two to three additional times.
5. If resin is to be reused, wash the resin five times with one bed volume of 3M NaSCN to remove bound Pierce c-Myc Peptide.

   Note: Pierce c-Myc Peptide may interfere with protein determination assays and absorbance at 280nm. Desalt sample before performing protein assay.

- **Chemical Elution Protocol:**

Note: Three options are available for chemical elution: 0.1M glycine, pH 2-2.8; 3M NaSCN; and 50mM NaOH (Table 2).

Table 2. Advantages and disadvantages of the chemical elution options.

Solution	Advantage	Disadvantage
0.1M Glycine, pH 2-2.8	• Useful if protein is resistant to low pH • Preserves resin binding activity	• May denature protein • Elution capacity is generally lower
50mM NaOH	• High elution capacity	• May denature protein • Reduces resin life
3M NaSCN	• High elution capacity • Preserves resin binding capacity	• May denature protein

Note: No loss of binding capacity occurs after 10 binding/elution steps of 0.1M glycine or 3M NaSCN; however, loss of resin activity can occur with exposure to 50mM NaOH.

1. Add one bed volume of either 0.1M glycine, pH 2.0-2.8, 50mM NaOH or 3M NaSCN to the column. Alternatively, the protein may be eluted by adding one bed volume of non-reducing 2X SDS-PAGE loading buffer.

   Note: Using 2X SDS-PAGE loading buffer will denature the anti-c-Myc antibody, which inactivates the resin.
2. Pellet resin with a 5-10 second pulse at 12,000 × *g*. Collect eluate. If using glycine or NaOH, neutralize the elution fraction with a 1:10-1:20 volume of 1M Tris, pH 9.5.
3. Repeat Steps 1 and 2 two additional times. Do not keep elution buffers on columns for extended periods of time.
4. If the resin is to be reused, wash the column with five bed volumes of 3M NaSCN, followed by 10 bed washes with TBS.

Procedure for Column Purification of c-Myc-tagged Protein

A. Column Set-up

1. Pre-equilibrate the resin and buffers and perform all steps at room temperature. If the protein is temperature-sensitive, the procedure may be performed at 4ºC.
2. Obtain a spin or gravity-flow column. The flow rate of the gravity flow column can be controlled by adding tubing at the bottom opening of the column. Use the recommended centrifuge force if using a spin column.
3. Resuspend resin and add 1-4mL of the slurry to the column. Allow the bed to drain. Wash the column with 2-5 bed volumes of TBS. Do not allow the resin to become dry.

B. Binding of c-Myc Fusion Protein to Column

1. Add cell lysate to column. Ensure lysate volume is at least equal to the bed volume. Adjust volume with TBS if needed.
2. Adjust the flow rate to 0.5mL/min. Multiple binding passes may be required for complete binding. Capping the column and incubating on an end-over-end rocker may improve binding.
3. Collect flow-through and save for analyzing binding efficiency.
4. Wash the column with 10 bed volumes of TBS-T. Washes can be analyzed by measuring the absorption at 280nm or by protein assay to confirm if the final washes contain no protein.

C. Elution of c-Myc Fusion Protein from Column

Note: Select one of the elution protocols below. If the eluted c-Myc-tagged protein will be used for function applications or is sensitive to pH extremes or sodium thiocyanate, then elute the protein with the Pierce c-Myc Peptide.

- **Gentle Elution Protocol:**

1. Add the bottom plug to the column and add one bed volume of 1mg/mL Pierce c-Myc Peptide in TBS. Incubate at 30ºC for 10-15 minutes. Elutions may be performed at lower temperatures, but elution efficiency may be reduced.
2. Remove column plug and cap and collect elution fraction.
3. Repeat Steps 1 and 2 two to three more times.
4. If the resin is to be reused, wash the column with five bed volumes of 3M NaSCN, followed by 10 bed washes of TBS.
5. For storage of the column, add two bed volumes of TBS containing 0.05% azide. Store column at 4ºC.

- **Chemical Elution Protocol:**

1. Add one bed volume of 0.1M glycine pH 2.0-2.8, 3M NaSCN, or 50mM NaOH three times.
2. Repeat Step 1 two additional times for a total of three elution fractions.
3. Collect elution fraction. If using glycine or sodium hydroxide elution, neutralize the fraction with 1:10-1:20 of 1M Tris, pH 9.5. Do not keep the elution buffers on the column for an extended period of time.
4. If the resin is to be reused, wash the column with five bed volumes of 3M NaSCN, followed by 10 bed washes of TBS.
5. For storage of the column, add two bed volumes of TBS containing 0.05% azide. Store column at 4ºC.

Troubleshooting

Problem	Possible Cause	Solution
c-Myc-tagged protein is in the flow-through	Column was overloaded	Reduce amount of lysate added to column
		Increase the amount of resin
	Fusion tag was not accessible to resin	Denature protein
		Switch c-Myc tag to the other terminus of the protein
	Column was not regenerated after use	Regenerate column with 3M NaSCN
Minimal or no c-Myc-tagged protein present in the elution fractions	Protein degraded	Perform purifications at 4ºC and include protease inhibitors during the binding step
	Protein was not fully eluted	Prepare additional elution fractions
		Use a different elution buffer (see Table 2 for recommendations)
	Protein was not expressed	Check protein lysate for presence of c-Myc-fusion protein by Western blot before purification
	Protein expression was very low	Add more lysate
		Optimize expression conditions
c-Myc-tagged protein appears as multiple bands on stained gels	Protease activity occurred during purification	Add protease inhibitors to lysate and wash buffers
	Wash step was insufficient	Add additional wash steps
		Increase detergent concentration in the wash buffer
		Increase sodium chloride concentration in the wash buffer
Elution with SDS-PAGE loading buffer produces multiple bands on stained gels	Reducing sample buffer was used and the antibody's 25kDa light chain and 50kDa heavy chain are visible	Omit reducing agent from the sample buffer

Related Thermo Scientific Products

26180	**Pierce c-Myc-Tag IP/Co-IP Kit**
26183	**Anti-c-Myc Antibody**, 100µg
20170	**Pierce c-Myc Peptide**, 5mg
87786	**Halt Protease Inhibitor Cocktail**, 1mL
69705	**Pierce Spin Columns – Screw Cap**, 0.9mL, 25 units
78260	**B-PER II Bacterial Protein Extraction Reagent**, 250mL
78501	**M-PER Mammalian Protein Extraction Reagent,** 250mL
89897	**Pierce Centrifuge Columns,** 5mL

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