

Pierce c-Myc Tag IP/Co-IP Kit

23620

1466.4

Number	Description
23620	<p>Pierce c-Myc Tag IP/Co-IP Kit, contains sufficient components for 25 immunoprecipitations or co-immunoprecipitations involving c-Myc-tagged proteins, including 10 experiments with the c-Myc-tagged Positive Control</p> <p>Kit Contents:</p> <p>Anti-c-Myc Agarose, 62.5µg settled beaded agarose coupled with 125µg of antibody and supplied as a 25% slurry (i.e., 250µL total volume) in PBS and 0.1% sodium azide as preservative</p> <p>BupH™ Tris Buffered Saline Pack, 1 pack, contains 25mM Tris, 0.15M NaCl, pH 7.2 when reconstituted with 500mL of ultrapure water</p> <p>Elution Buffer (pH 2.0), 2 × 25mL</p> <p>Lane Marker Non-Reducing Sample Buffer (5X), 5mL, contains 0.3M Tris•HCl, pH 6.8, 5% SDS, 50% glycerol, lane marker tracking dye</p> <p>Pierce Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps</p> <p>Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps</p> <p>Conditioning Buffer (100X), 5mL, neutral-pH buffer</p> <p>c-Myc-tagged Positive Control, 500µL, 1mg/mL <i>E. coli</i> extract containing c-Myc-tagged GST (Glutathione S-Transferase)</p>

Storage: Upon receipt, store the c-Myc-tagged Positive Control at -80°C (stable for six months at -20°C). Store Application Set at 4°C. Application Set is shipped at ambient temperature. The c-Myc-tagged Positive Control is shipped with dry ice.

Note: The c-Myc Tag IP/Co-IP Kit is a combination of the c-Myc Tag IP/Co-IP Application Set (Product No. 23622) and the c-Myc-tagged Positive Control (Product No. 23633).

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Introduction

The Thermo Scientific™ Pierce™ c-Myc Tag IP/Co-IP Kit provides a simple and fast method to study c-Myc-tagged proteins, with advantages over the traditional IP procedure using protein A/G agarose. The high affinity anti-c-Myc antibody-coupled agarose enables immunoprecipitation (IP) of c-Myc-tagged proteins or co-immunoprecipitation (Co-IP) of their interacting partners without antibody contamination. The Pierce Spin Columns provided with this product handle small volumes of anti-c-Myc agarose and allow the entire procedure to be performed in a single spin column, preventing anti-c-Myc agarose loss between washes. The spin columns further enhance recovery by efficiently separating buffer from the anti-c-Myc agarose.¹

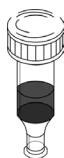
Epitope tagging enables surveillance of the resultant fusion protein with tag-specific rather than protein-specific antibodies.^{2,3} Immunoprecipitation of epitope-tagged proteins can be performed to determine the proteins cellular localization, to study post-translation modifications or to detect interactions between tagged proteins and other proteins. The c-Myc tag consists of ten amino acids (EQKLISEEDL) derived from the C-terminus of human c-Myc protein. Immunoprecipitation of c-Myc-tagged proteins using anti-c-Myc antibodies distinguishes the expressed protein from the native protein population.

The c-Myc-Tag IP/Co-IP Kit uses a high-affinity anti-c-Myc antibody that enables the isolation and identification of tagged protein regardless of their expression level. The covalent linkage between the antibody and the agarose results in a final immunoprecipitated product free of antibody contamination. The c-Myc-tagged Positive Control can be used to verify IP and anti-c-Myc agarose performance. The spin column format facilitates complete retention of the anti-c-Myc agarose during the IP/Co-IP and minimizes variability between experiments that result from the loss of anti-c-Myc agarose (See Appendix for a diagram of the spin columns and data relating to kit performance).

Procedure Summary



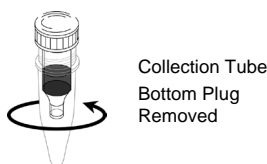
1. Lyse cells expressing c-Myc-tagged protein.



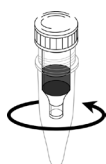
2. Combine cell lysate and immobilized Anti-c-Myc (anti-c-Myc agarose).



3. Incubate at 4°C for 1 hour to overnight with end-over-end mixing.



4. Pulse centrifuge for 10 seconds.



5. Wash three times with TBS-T. Pulse centrifuge for 10 seconds after each wash.



6. Elute c-Myc-tagged protein using Elution Buffer or Non-Reducing Sample Buffer.

Important Product Information

- For best results, determine optimal conditions for expression of c-Myc-tagged fusion protein before attempting IP.
- Include a non-transfected lysate as a negative control to identify nonspecific binding of proteins to the anti-c-Myc agarose. The c-Myc-tagged Positive Control (provided with the c-Myc-tag IP/Co-IP Kit) assists in verifying whether the anti-c-Myc agarose can successfully capture the c-Myc-tagged protein.
- The buffer provided in the kit allows complete flexibility to determine optimal conditions for isolating interacting proteins. Characteristics of interacting proteins may require alteration of the binding and wash buffers. Protein cofactors, divalent cations, detergents or additional salts may be added.
- For optimal results in obtaining c-Myc-tagged proteins, add protease inhibitors (e.g., Halt™ Protease Inhibitor Cocktail, EDTA-Free, Product No. 87785) when preparing any lysate.

Additional Materials Required

- Microcentrifuge capable of $16,000 \times g$
- Protease inhibitors (Halt Protease Inhibitor Cocktail, EDTA-Free, Product No. 87785)
- Tween™ 20 (Surfact-Amps™ 20, Product No. 28320)
- End-over-end rocker or rotator
- Heat block
- 0.2µm, 500mL filter apparatus
- 1 M Tris, pH 9.5
- 1 M Dithiothreitol (DTT, Product No. 20290) or 2-mercaptoethanol (2-ME, Product No. 35602)

Material Preparation

- **BupH Tris Buffered Saline Pack (TBS):** Reconstitute contents with 500mL of ultrapure water. Filter sterilize solution using a 0.2µm filter apparatus and store at 4°C. Final concentration is 25mM Tris•HCl, 0.15M NaCl; pH 7.2.
- **Non-Reducing Sample Buffer (2X):** Equilibrate the Lane Marker Non-Reducing Sample Buffer (5X) to room temperature. Gently mix Sample Buffer by inverting the bottle 5-10 times. The Sample Buffer is viscous and may require that the pipette tip be “snipped” to allow the solution to be drawn into the tip. Dilute Sample Buffer by adding 600µL ultrapure water to 400µL Sample Buffer. Pipette up and down to mix. Store solution at 4°C for up to one year.

Procedure for IP of c-Myc-tagged protein

A. Immunoprecipitation/Co-immunoprecipitation (IP/Co-IP)

Note: The lysate amount needed and incubation time are dependent upon the expression level of the c-Myc-tagged protein and will have to be optimized for each specific system. For Co-IP experiments, the buffer system must to be optimized to maintain the protein:protein interaction.

1. Place the bottom plugs on the Pierce Spin Columns. Add the appropriate amount of lysate to the spin columns.
Note: For a well expressed tagged protein, using 200µL lysate (400-600µg total protein) from a 60mm plate confluent cells is a good place to start for optimization (see Figure 1). The maximum volume for the spin column is 850 µL.
2. To set up a positive control, use 50µL c-Myc-tagged Positive Control diluted in 150µL TBS.
3. Thoroughly resuspend the anti-c-Myc agarose by inverting the vial several times immediately before dispensing (do not vortex!). Dispense 10µL anti-c-Myc agarose slurry (5µg anti-c-Myc antibody) into each labeled spin column using a wide-bore pipette tip. Screw on the cap.
4. Incubate with gentle end-over-end mixing for at least 1 hour at 4°C, typical incubation times range from 2 hours to overnight.
5. Prepare a wash solution of TBS plus 0.05% Tween 20 (TBS-T). For each spin column prepare approximately 3mL of wash solution.
6. Loosen the top cap on the column, and then remove the bottom plug. Put a collection tube under the column and pulse centrifuge for 10 seconds. Discard the flow-through (or save for future analysis).
7. Add 0.5mL of TBS-T to each column. Loosely screw on the cap and gently invert the column with the collection tube 2-3 times. Pulse centrifuge for 10 seconds. Discard the wash (or save for future analysis). Repeat this step two additional times.
8. Wash the resin once with 500µL of 1X Conditioning Buffer.

B. Elution of c-Myc-tagged Protein

Note: If the eluted c-Myc-tagged protein will be used for functional applications, use Elution Protocol 1 to elute the protein. If the protein is sensitive to the low pH, use Gentle Elution Buffer (Product No. 21027). For electrophoretic analysis, use Elution Protocol 2.

• Elution Protocol 1:

1. Place the spin column in a new collection tube. Add 10 μ L Elution Buffer to the anti-c-Myc agarose, loosely screw on the cap and gently tap the tube to mix. Pulse centrifuge for 10 seconds.

Note: It is not necessary to place the bottom plug on the spin column for this step.

2. Repeat step 1 two additional times. The three elutions may be recovered and pooled in one collection tube.

Note: Neutralize the eluent immediately by adding 1 μ L of 1M Tris, pH 9.5 per 20 μ L of Elution Buffer.

3. For reducing gel analysis, prepare reducing sample buffer by adding 10 μ L of 1M DTT or 5 μ L of 2-mercaptoethanol to the 40 μ L of Lane Marker Non-Reducing Sample Buffer (5X).
4. Add 7.5 μ L of the above-described reducing sample buffer to 30 μ L elution sample. Heat the sample at 95-100 $^{\circ}$ C for 5 minutes.

• Elution Protocol 2:

1. Place the spin column in a new collection tube. Add 25 μ L 2X Non-Reducing Sample Buffer, prepared from the 5X Sample Buffer, to the anti-c-Myc agarose, loosely screw on the cap and gently tap the tube to mix.

Note: It is not necessary to place the bottom plug on the spin column for this step.

2. Heat spin column/collection assembly at 95-100 $^{\circ}$ C on a heat block for 5 minutes. Pulse centrifuge for 10 seconds.

Note: Using Non-Reducing Sample Buffer can minimize interference from co-eluting antibody fragments.

3. To prepare the sample for reducing SDS-PAGE, add 2-3 μ L of 1M DTT or 1-2 μ L of 2-ME to the 25 μ L sample.

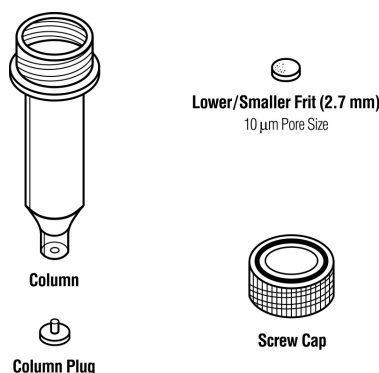
Note: The eluted c-Myc-tagged Positive Control can be detected by coomassie or silver staining (see Related Thermo Scientific Products). For more sensitive detection methods such as Western blotting, dilute the eluted c-Myc-tagged Positive Control 10- to 50-fold (0.2-0.5 μ L elution is usually sufficient for analysis).

Troubleshooting

Problem	Cause	Solution
Minimal or no c-Myc-tagged protein is detected	Degraded tagged protein	Include protease inhibitors in the lysis buffer Use new lysate or lysate stored at -80 $^{\circ}$ C
	No or minimal tagged protein expression	Verify protein expression by SDS-PAGE or Western blot analysis of the lysate using c-Myc-tagged Positive Control as a reference Increase the amount of lysate used for IP/Co-IP
	Insensitive detection system	Use more sensitive detection system such as SuperSignal TM West Femto Chemiluminescent Substrate* (Product No. 34095)
	Failure to Co-IP interacting protein	Wash conditions too stringent for the weak or transient interaction Reduce the number of washes and/or lower the ionic strength of wash buffer
Failure to Co-IP interacting protein	Low expression level of interacting protein	Apply additional protein sample or use a more sensitive detection system
	Inappropriate buffer system to retain the protein:protein interaction	Optimize the Co-IP buffer

Appendix

A. Diagram of the Pierce™ Spin Column



B. Immunoprecipitation of c-Myc-tagged E2_{EPF}

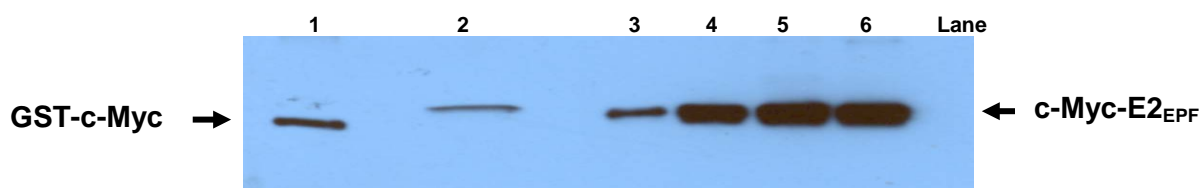


Figure 1. Immunoprecipitation of c-Myc-tagged E2_{EPF} from a mammalian cell lysate. Human embryonic kidney cells (293) were transfected with pCMV-c-Myc-E2_{EPF} for 40 hours. Cells were then lysed in M-PER™ Mammalian Protein Extraction Reagent (200μL/60mm plate) containing protease inhibitors and 200μL of the lysate (~500μg total protein) was incubated with 2, 5, 10 and 20μL (Lane 3, 4, 5 and 6) anti-c-Myc agarose slurry at 4°C overnight. Anti-c-Myc agarose slurry (10μL) was incubated with 50μL positive control lysate from the kit (Lane 1). c-Myc-tagged proteins were eluted with 25μL non-reducing sample buffer, and 10μL of each eluted sample was separated by SDS-PAGE, transferred to a nitrocellulose membrane and probed with anti-c-Myc antibody. Goat Anti-Mouse IgG (H+L), Peroxidase Conjugated (Product No. 31430; 1:100,000 dilution) was used as the secondary antibody. The membrane was incubated with SuperSignal West Pico Chemiluminescent Substrate (Product No. 34080) and exposed to X-ray film. The expression of c-Myc-E2_{EPF} was verified by applying 15μL transfected cell lysate (Lane 2) on the same gel.

C. Co-immunoprecipitation of LTA (SV40 Large T-antigen) with c-Myc-tagged p53

The tumor suppressor p53, forms a complex with SV40 (DNA tumor virus) large T antigen. This association increases the stability of p53 protein and renders p53 incapable of binding to DNA and inducing transcription. Anti-c-Myc antibody can IP c-Myc tagged p53 (Figure 2, Lane 4) and Co-IP LTA with c-Myc-tagged p53 (Lane 3). There is no nonspecific binding on the antibody (Lane 5) and agarose resin (Lane 6).

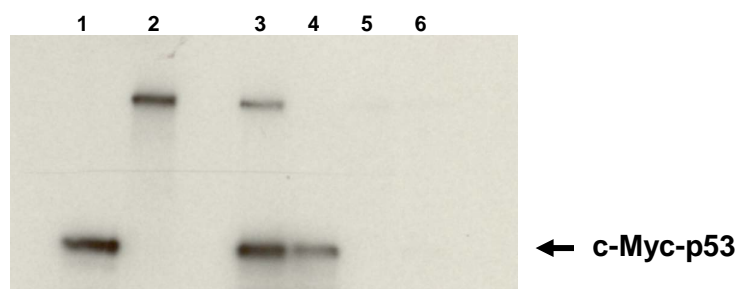


Figure 2. Co-immunoprecipitation of LTA with c-Myc-tagged p53. LTA and c-Myc-p53 were expressed *in vitro*, and ³⁵S-labeled (Lane 1 and 2) using TNT™ Coupled Reticulocyte Lysate System. Before IP and Co-IP, the lysates (5μL each) of LTA and c-Myc-p53 (Lane 3 & 6), c-Myc-p53 (Lane 4) alone or LTA alone (Lane 5) were incubated at 30°C for 1 hour, and 10μL of anti-c-Myc agarose slurry (Lane 3, 4 and 5) or plain agarose slurry (Lane 6) was added to the corresponding sample. IP and Co-IP reactions were performed at 4°C overnight. IP and Co-IP products were eluted with 25μL non-reducing sample buffer and separated on 12% SDS-PAGE. The ³⁵S-labeled proteins were detected by fluorography.

Related Thermo Scientific Products

20168	Pierce Anti-c-Myc Agarose, 2mL
20169	Pierce Anti-c-Myc Agarose, 10mL
88844	Pierce c-Myc Tag Magnetic IP/Co-IP Kit
88842	Pierce Anti-c-Myc Magnetic Beads, 1mL
88843	Pierce Anti-c-Myc Magnetic Beads, 5mL
82033	Pierce Agarose qIP Protein Interaction Kit, Tluc and Myc tags
82036	Pierce Magnetic qIP Protein Interaction Kit, Tluc and Myc tags
87785	Halt Protease Inhibitor Cocktail, EDTA-Free (100X), 1mL
69705	Pierce Spin Columns and Accessories, 25 units
21027	Gentle Ag/Ab Elution Buffer, 500mL
35602	2-Mercaptoethanol, 10 × 1mL
20290	DTT, Cleland's Reagent (Dithiothreitol), 5g

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