

INSTRUCTIONS



Pierce[®] IP Lysis Buffer

87787 87788

2247.0

Number Description

87787 Pierce IP Lysis Buffer, 100 ml

87788 Pierce IP Lysis Buffer, 250 ml

Contents: 25 mM Tris•HCl pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 5% glycerol

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce IP Lysis Buffer is a mammalian whole cell lysis buffer based on a modified RIPA buffer formulation without SDS. This moderate strength lysis buffer is able to effectively solubilize cellular proteins but does not liberate genomic DNA or disrupt protein complexes like RIPA buffer. The Pierce IP Lysis Buffer is especially formulated for pulldown and immunoprecipitation assays and as a wash buffer for affinity resins. This lysis buffer is also compatible with many other applications, including protein assays, protein purification and immunoassays (e.g., ELISA, Western blot).

Important Product Information

- Pierce IP Lysis Buffer does not contain protease or phosphatase inhibitors. If desired, add Thermo Scientific Halt Protease and Phosphatase Inhibitor Cocktail (Product No. 78440) or Halt Protease Inhibitor Cocktail (Product No. 87786) immediately before use.
- Pierce IP Lysis Buffer has been tested on representative cell types including the following cell lines: HeLa, Jurkat, A431, A549, MOPC, NIH 3T3, HepG2, COS-7 and U2OS. Typically, 10⁶ HeLa cells yield ~10 mg of cell pellet and ~3 µg/µl (or 300 µg) using 100 µl of lysis buffer.
- To use Pierce IP Lysis Buffer with the Thermo Scientific Active GTPase Pull-Down and Detection Kits, add MgCl₂ to a final concentration of 6 mM to neutralize EDTA. Some reporter assays, such as luciferase, might require additional cofactors.
- Pierce IP Lysis Buffer is compatible with the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225) and Pierce 660 nm Protein Assay (Product No. 22660).

Procedure for Lysing Cell Monolayer (Adherent) Cultures

1. Carefully remove culture medium from cells. Wash the cells once with ice cold phosphate-buffered saline (PBS; 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2; Product No. 28372).
2. Add ice cold lysis buffer to the cells according to the table below and incubate on ice for 5 minutes with periodic mixing.

Plate Size/Surface Area	Volume of Pierce IP Lysis Buffer
100 × 100 mm	500-1,000 µl
100 × 60 mm	250-500 µl
6-well plate	200-400 µl per well
24-well plate	100-200 µl per well

3. Transfer the lysate to a microcentrifuge tube and centrifuge at ~13,000 × g for 10 minutes to pellet the cell debris at 4°C.
4. Transfer supernatant to a new tube for protein concentration determination and further analysis.

Procedure for Lysing Cell Suspension Cultures

1. Centrifuge the cell suspension at $1,000 \times g$ for 5 minutes to pellet the cells. Discard the supernatant.
2. Wash the cells once with ice cold PBS. Centrifuge at $1,000 \times g$ for 5 minutes to pellet cells.
3. Add ice cold Pierce IP Lysis Buffer to the cell pellet. Use 500 μl of lysis buffer per 50 mg of wet cell pellet (10:1 v/w). **Note:** If using a large amount of cells, first add 10% of the final volume of lysis buffer to the pellet and pipette the mixture up and down to mix. Add the remaining volume of lysis buffer to the cell suspension.
4. Incubate lysate on ice for 5 minutes with periodic mixing. Remove cell debris by centrifugation at $\sim 13,000 \times g$ for 10 minutes at 4°C .
5. Transfer supernatant to a new tube for protein concentration determination and further analysis.

Troubleshooting

Problem	Possible Cause	Solution
Low total protein yield	Some cells are more resistant to lysis than others	Thoroughly suspended the cell pellet in buffer and incubate for longer with occasional mixing
Low concentration of proteins	Excess buffer used	Use less buffer
Proteolysis	No protease inhibitors added	Add protease inhibitors to the lysis buffer before use
Lack of phosphorylated proteins	Phosphatase activity	Add phosphatase inhibitors to the buffer before use
	Protein is non-phosphorylated	None

Related Thermo Scientific Products

87786	Halt Protease Inhibitor Cocktail (100X), 1 ml
78440	Halt Protease and Phosphatase Inhibitor Cocktail (100X), 1 ml
89901	RIPA Buffer, 250 ml
78501	M-PER® Mammalian Protein Extraction Reagent, 250 ml
78510	T-PER® Tissue Protein Extraction Reagent, 500 ml
23227	Pierce BCA Protein Assay Reagent Kit
22660	Pierce 660 nm Protein Assay Reagent, 750 ml
28372	BupH™ Phosphate Buffered Saline (PBS), 40 packs each pack makes 500 ml
22811	Pierce Protein A Plus Agarose, 5 ml
22851	Pierce Protein G Plus Agarose, 2 ml
20423	Pierce Protein A/G Plus Agarose, 2 ml

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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