INSTRUCTIONS

Pierce Protein Transfection Reagent Kit

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89850

Number	Description
89850	**Pierce Protein Transfection Reagent Kit**, sufficient reagent for 24 reactions using a 6-well plate or 35mm dish or 96 reactions using a 24-well plate

Kit Contents:

89850A	**Pierce Protein Transfection Reagent,** supplied as dried film
89850B	**Pierce β-Galactosidase Control**, 100µL, 0.1µg/µL
89850C	**Pierce FITC-Antibody Control**, 100µL, 0.1µg/µL

Storage: Upon receipt store at -20°C. Product is shipped on dry ice.

Note: For optimal results, do not repeatedly freeze-thaw the β-galactosidase and FITC-antibody controls. Store the control proteins in aliquots suitable for one experiment.

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Introduction

The Thermo Scientific™ Pierce™ Protein Transfection Reagent is a unique cationic lipid-based carrier system[1-3] that can be used to deliver biologically active proteins, peptides or antibodies into living cells. Pierce Reagent/protein complexes attach to negatively charged cell surfaces and enter the cell either by directly fusing with the plasma membrane or by endocytosis and subsequent fusion with the endosome, releasing the captured protein into the cytoplasm.[4] Pierce Protein Transfection Reagent can deliver various molecules over a broad range of cell types in serum-free conditions. Proteins delivered into the cells remain biologically active because the Pierce Reagent/protein interaction is noncovalent. Therefore, Pierce Reagent may be used in applications relevant to intercellular signaling, cell cycle regulation, apoptosis, oncogenesis and transcriptional regulation.

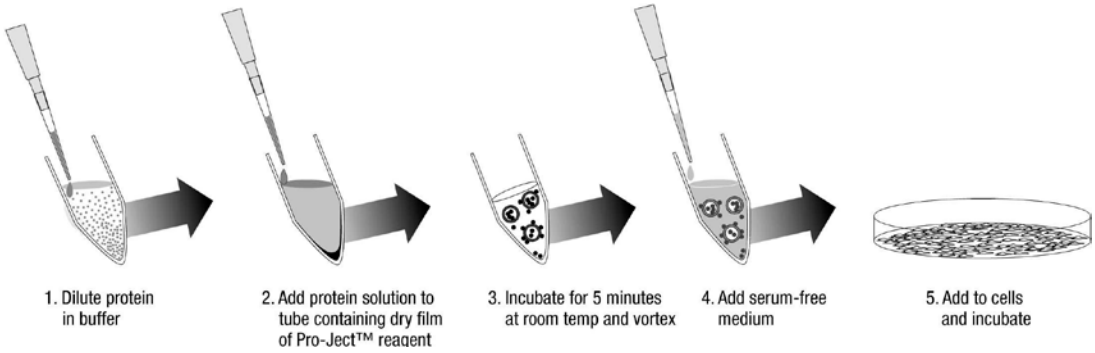
Advantages of using this system are as follows:

- Easy to use
- Non-cytotoxic
- More economical than microinjection and electroporation
- Faster than introducing transcriptionally active DNA into cells and subsequently expressing the protein *in vivo*

Molecules that have been successfully delivered with Pierce Protein Transfection Reagent include β-galactosidase, fluorescent-antibody, green fluorescent protein (GFP), high and low molecular weight dextran sulfate, phycoerythrin-BSA, caspase 3, caspase 8 and granzyme B.

Procedure Summary for General Use

Total assay time: 2 ½ -4 ½ hours



Procedure for General Use of the Pierce Protein Transfection Reagent

Preparation of the Pierce Protein Transfection Reagent

1. Dissolve the Pierce™ Reagent by adding 250 µl of methanol or chloroform to the tube containing the dry film.
2. Vortex for 10-20 seconds at top speed before each use.

   Note: Handle chloroform with caution. Wear appropriate protective clothing and use a well-ventilated space or fume hood. Keep chloroform containers closed to avoid excessive evaporation.
3. Pipette the amount of Pierce Reagent into a microcentrifuge tube as indicated in Table 1. Dispense the solution to the bottom of the tube. The amount of Pierce Reagent necessary depends on specific experimental parameters (i.e., cell type, assay sensitivity, plate size, etc.). To optimize the delivery conditions, vary the amount of protein/peptide to be delivered first; then vary the amount of Pierce Reagent, if necessary. Begin by using 10µL of Pierce Reagent per reaction in a 6-well plate or 2.5µL for a 24-well plate and vary the amount of protein to be delivered.

Table 1. Suggested volume of Pierce Reagent to use for various assays.

Tissue Culture Dish	Pierce Reagent Volume (µL)	Number of Reactions/Kit
96-well	1	240
24-well	2.5	96
12-well	5	48
6-well	10	24
60 mm dish	20	12
100 mm dish	35	7

4. Evaporate the solvent by placing the microcentrifuge tubes containing the Pierce Reagent under a laminar flow hood for a minimum of 2 hours at room temperature. For larger volumes, evaporate for a minimum of 3-4 hours. Alternatively, use an inert gas such as argon or nitrogen to quickly evaporate the solvent. Apply the gas flow gradually to avoid splashing the Pierce Reagent off the bottom of the tube. Optionally, vacuum dry the Pierce Film for 1-2 additional hours to completely remove solvent.

   Note: The quality of the protein delivery reaction is adversely affected by the presence of methanol or chloroform. Follow the recommended 2- to 4-hour drying time to ensure removal of all traces of solvent before adding protein or peptide.
5. Proceed to the next step (B. Preparation of Pierce Reagent/Protein Complexes) or store the closed tubes at -20°C until use.

   Note: To prevent extensive evaporation of the solvent and variations in reagent concentration, aliquot the Pierce Reagent immediately after dissolving in 250µL chloroform or methanol. Dried Pierce Reagent can be stored at -20°C for at least one year without any substantial loss of activity.

Preparation of Pierce Reagent/Protein Complexes

Experimental results suggest that highly positively charged molecules interact poorly with the Pierce Reagent and, therefore, are not delivered into cells efficiently.

6. Dilute the protein, peptide or other macromolecule in one of the following buffers:
   - HEPES Buffered Saline (HBS; 10mM HEPES, 150mM NaCl, pH 7.0)
   - Phosphate Buffered Saline (PBS; 20mM Na phosphate, 150mM NaCl, pH 7.4)
   - Tris Buffered Saline (TBS; 10mM Tris, 150mM NaCl, pH 7.0)

7. The final concentration of the protein, peptide or macromolecule of interest is dependent upon its intrinsic properties and the type of assay performed. Refer to Table 2 for guidelines.

Table 2. Protein concentration ranges used successfully for delivery.

Protein	Concentration Range
Antibody, β-galactosidase or dextran sulfate	50-250 µg/mL
Caspase3	0.05 to 0.3 units/µL (165 to 1,000 pg/µL)
Granzyme B	7.5 to 60 ng/µL

8. Hydrate the dried Pierce Reagent with the diluted protein solution. Pipette up and down 3-5 times. Incubate at room temperature for 5 minutes. Vortex for 3-5 seconds at low to medium speed. The amount of protein, peptide, antibody or other molecule to be delivered is dependent on the type of experiment (cell type, assay sensitivity, plate size, etc.). Refer to Table 3 for guidelines.

Table 3. Suggested quantity of proteins and hydration volumes.

Tissue Culture Dish	Protein/ Ab, β-gal (µg)	Protein/ Caspase3 (ng)	Protein/ Granzyme (µg)	Pierce Reagent Hydration Volume (µL)
96-well	0.1-0.25	0.25-0.5	0.01-0.05	10
24-well	1-2	2-4	0.075-0.5	10-25
12-well	2-4	4-8	0.15-1	25-50
6-well	5-10	10-20	0.3-2	50-100
60mm dish	10-20	20-40	0.5-3	100-400
100mm dish	25-50	50-100	0.75-4	250-500

9. Add serum-free medium to the Pierce Reagent/protein complex to bring the final delivery volume up to the amounts recommended in Table 4.

Table 4. Suggested cell number and transfection volume.

Tissue Culture Dish	Number of Cells	Total Delivery Mix Volume
96-well	1-2 × 10^4	100µL
24-well	0.5-1 × 10^5	250µL
12-well	1-2 × 10^5	500µL
6-well	2-4 × 10^5	1mL
60 mm dish	5-10 × 10^5	2.5mL
100 mm dish	10-20 × 10^5	5mL

10. Aspirate the medium from the cells to be tested. Wash cells once with serum-free medium (optional). Transfer the final delivery mix (from step B.4) onto the cells.
11. For adherent cells, directly add the Pierce Reagent/protein complexes (resuspended in serum-free medium) onto the washed cells. Proceed to step B.7.

    For suspension cells, count cells and centrifuge them at 1,200 rpm (300 x *g*) for 5 minutes. Resuspend cells in serum-free medium. Adjust the concentration according to the size of plate or dish being used and the corresponding transfection volume. Pipette the cell suspension into the tube of Pierce Reagent/protein mixture and then transfer to a plate or dish.
12. Incubate for 3-4 hours at 37°C. If a longer incubation time is required, add one volume of 20% serum-containing medium directly to the well or dish. It is not necessary to change the medium after the initial serum-free incubation. However, if incubation times longer than 24 hours are necessary, medium can be replaced as needed.

    Note: The presence of serum in the first hours of incubation is inhibitory for delivery. Ensure that the first 3-4 hours of incubation are performed in serum-free conditions followed by growth in serum-containing medium.
13. Proceed with the experiment for observation or detection assays. Cells can be fixed or can be observed live.

    Note: Two positive controls are provided in the kit. The fluorescein-antibody and β-galactosidase may be tested as described in the example protocols.

Example Protocol for Delivery of a Fluorescent Antibody or β-Galactosidase using a 6-well plate or 35 mm dish

1. Seed 2 × 10^5 cells in a 6-well plate and let grow overnight.
2. Pipette 10µL of Pierce Reagent (dissolved in 250µL of methanol or chloroform) into the bottom of each microcentrifuge tube. Evaporate the solvent under a laminar flow hood for 2 hours (or blow dry with an inert gas). Optionally, vacuum the dried Pierce Film for 2 additional hours to completely remove any traces of solvent. Proceed to the next step or store the tubes at -20°C until use.
3. Dilute 5-10µg of protein in 50-100µL of HBS or PBS (refer to step B.1.). PBS is recommended for β-galactosidase. The FITC-Ab and β-galactosidase control proteins provided in the kit are ready to use without further manipulation. Thaw and mix well before use.
4. Hydrate the dried Pierce™ Film with 50-100µL of the diluted protein solution. Pipette up and down 3-5 times. Incubate at room temperature for 5 minutes. Vortex for a few seconds at low to medium speed.
5. Bring the final volume of the Pierce Reagent/protein mixture to 1,000µL with serum free medium.
6. Aspirate the medium from the cells to be tested, wash one time with serum-free medium (optional). Transfer the Pierce Reagent/protein mixture directly onto the cells.
7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. If incubation time is to be longer than 4 hours, add 1,000µL (one volume) of 20% serum-containing medium directly to the well.

8. Wash the cells twice with PBS. Proceed with the appropriate assay.

   Fluorescent microscopy: After washing, overlay cells with 1,000µL PBS. Observe living cells directly under a microscope. Alternatively, fix cells before observation.

   β-galactosidase assay: Proceed to Example Protocol for Staining of β-galactosidase.

Example Protocol for Delivery of a Fluorescent Antibody or β-Galactosidase using a 24-well plate

1. Seed 0.5 to 1 × 10^5 cells per well in a 24-well plate or on a cover slip. Let grow overnight.
2. Pipette 2.5µL of Pierce Reagent (dissolved in chloroform or methanol) into the bottom of each microcentrifuge tube. Evaporate the solvent under a laminar flow hood for 2 hours (or blow dry with an inert gas). Optionally, vacuum the dried Pierce Film for 2 additional hours to completely remove any trace amount of solvent. Proceed to the next step or store the tubes at -20°C until use.
3. Dilute 0.5-2.0µg of FITC-Ab or β-galactosidase in 10-25µL of HBS or PBS (refer to step B.1.). PBS is recommended for β-galactosidase. The FITC-Ab and β-galactosidase control proteins provided in the kit are ready to use without further manipulation. Thaw and mix them well before use.
4. Hydrate the dried Pierce Film with 10-25µL of the diluted protein solution. Pipette up and down 3-5 times. Incubate at room temperature for 3-5 minutes. Vortex for a few seconds at low to medium speed.
5. Bring the final volume of the Pierce Reagent/protein mixture to 250µL with serum-free medium.
6. Aspirate the medium from the cells to be tested, wash once with serum-free medium (optional). Transfer the Pierce Reagent/protein mixture directly onto the cells.

   Note: If cover slips are used, blot the cover slip dry and place it in a 35-mm dish. Transfer the Pierce Reagent/protein mixture directly onto the cells.
7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. Add 250µL (one volume) of 20% serum-containing medium directly to the 24-well plate if the incubation time needs to be longer than 4 hours.
8. For cover slips, add 1-2mL of growth medium to the 35-mm dish containing the cover slip if the incubation time will be longer than 4 hours.
9. After the incubation, wash the cells twice with PBS and proceed with the appropriate assay.

   Fluorescent microscopy: After washing, mount cells growing on cover slips directly onto a hanging drop slide with PBS. For 24-well plates, overlay cells with 250µL PBS. Observe living cells directly under a microscope. Alternatively, fix cells prior to observation.

   β-galactosidase assay: Proceed to Example Protocol for Staining of β-galactosidase.

Example Protocol for Staining β-Galactosidase

Note: Any histochemical staining procedure for β-galactosidase found in the literature is applicable.[5,6] Listed below is one example protocol.[6]

Additional Materials Required

- 10X Phosphate Buffered Saline (PBS; refer to step B.1.), store at room temperature
- 150 mM potassium ferricyanide, store at -20°C
- 150 mM potassium ferrocyanide, store at -20°C
- 20 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) resuspended in DMF (*N*, N-Dimethyl-formamide), store at -20°C in the dark
- 4% Paraformaldehyde in PBS, store at -20°C

1. Remove the medium from the transfected cells.
2. Wash the cells twice with 1X PBS.
3. Fix cells with 4% paraformaldehyde for 10 minutes at room temperature.
4. Prepare the Staining Solution according to Table 5. Staining Solution should be made immediately before use.

Table 5. Preparation of Staining Solution

Volume	Component	Final Concentration
250µL	150mM potassium ferricyanide	1.5mM
250µL	150mM potassium ferrocyanide	1.5mM
1.25mL	X-gal (20mg/mL in DMF)	1mg/mL final
23.25mL	1X PBS	
25mL Total Volume		

5. Wash cells twice with 1X PBS.
6. Add the Staining Solution to the cells.
7. Incubate the cells at 37°C for 2-8 hours.
8. Wash cells once with 1X PBS.
9. Observe the cells under the microscope.
10. Calculate the percent of cells transfected with β-galactosidase:

$$\% \text{ transfection} = \frac{\text{Total number of blue cells}}{\text{Total number of cells}} \times 100$$

Troubleshooting

Problem	Cause	Solution
Low delivery efficiency	Incomplete solubilization	Use chloroform or methanol to solubilize Pierce Reagent and vortex vigorously
	Incorrect aliquoting of the Pierce Reagent	Aliquot the Pierce Reagent to the bottom of the microcentrifuge tube
	Insufficient drying time	Use sufficient time to air dry or vacuum dry Pierce Reagent **Note**: If using inert gas to dry the reagent, ensure that Pierce Reagent does not splash onto the side of the tube
	Insufficient amount of Pierce Reagent	Vary the amount of reagent as recommended in the optimization protocol
	Incorrect protein/peptide concentration	Titrate the concentration and the hydration volume of Pierce Reagent
	Problems with hydration buffers	Change the protein dilution buffer and/or the pH to improve the delivery

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Problem	Cause	Solution
Low delivery efficiency	Incomplete mixing of Pierce Reagent and protein	Allow the mixtures to form for a minimum of 3 minutes **Note**: Mix by pipetting up and down; do not vortex *vigorously* at this step
	Highly positively charged molecules are difficult to deliver with Pierce Reagent	Modify the hydration buffer or pH to change the charge of the molecules
	Unknown properties of molecules to be delivered	Mix a fluorescent molecule or directly label the protein of interest in order to monitor delivery
	Large cell density	Use cells that are 50-60% confluent
	Incorrect medium used	Use serum-free medium during the first hours of delivery
	Improper storage of Pierce Reagent	Pierce Reagent is stable but long exposure to elevated temperatures may cause degradation
	Insufficient incubation time	Incubate Pierce Reagent/protein complexes with cells for at least 3-4 hours.
	Type of cell line used	Test Pierce Reagent with the positive controls in parallel with cell lines that were successfully used (refer to Table 1 for cell line suggestions)
Aggregation	Excess Pierce Reagent used	Lower the amount of Pierce Reagent used
	Concentration of the dissolved Pierce Reagent stock solution has changed due to excessive evaporation	Titrate down or use lower amounts of Pierce Reagent
	Improper storage of the Pierce Reagent/protein complexes	Pierce Reagent/protein complexes should be prepared immediately before use
Cytotoxicity	Excess Pierce Reagent	Decrease the amount of reagent used
	Molecules delivered were toxic	Use the appropriate control reactions such as cells alone, Pierce Reagent alone, “control” or “safe” protein alone, or the molecule(s) of interest alone Check the purity of the molecule of interest to be delivered
	Unhealthy cells	Check cells for contamination Thaw a new batch of cells Cells are too confluent or cell density is too low Check the culture medium (pH, kind used, last time changed, etc.) Check materials used for proper function (culture plates, incubator temperatures, etc.)

Related Thermo Scientific Products

Number	Description
53027	**Pierce™ FITC Antibody Labeling Kit**
53029	**Pierce™ NHS-Fluorescein Antibody Labeling Kit**

References

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2. Felgner, J.H. *et al*. (1994). Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J. Biol. Chem.* **269(4):**2550-61.
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Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | Thermofisher.com