

Pierce qIP Protein Interaction Tluc Assay Reagents

82015 82016

2560.2

Number	Description
82015	Pierce qIP Protein Interaction Tluc Assay Reagent Set , 150 luciferase assay reactions, store at -80°C: Pierce qIP Protein Interaction Tluc Assay Buffer , 10mL Coelenterazine Substrate (100X) , 2 amber vials (50µL/vial)
82016	Pierce qIP Protein Interaction Tluc Assay Reagent Set , 1500 luciferase assay reactions, store at -80°C: Pierce qIP Protein Interaction Tluc Assay Buffer , 100mL Coelenterazine Substrate (100X) , 2 amber vials (500µL/vial)

Storage: Upon receipt, store kit components at -80°C. The Pierce qIP Assay Reagents are shipped on dry ice.

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Introduction

The Thermo Scientific™ Pierce™ Quantitative Immunoprecipitation (qIP) Protein Interaction Kit provides a highly sensitive luciferase-based assay to measure protein-protein interactions in mammalian cells without Western blotting. The qIP assay utilizes the smallest and one of the brightest luciferases, Thermo Scientific™ TurboLuc™ (Tluc) Luciferase, fused to a protein of interest that is transiently co-expressed with an epitope-tagged protein in mammalian cells. The protein interactions are quantified by measuring Tluc Luciferase activity following pull-down of epitope-tagged proteins with anti-epitope agarose or magnetic beads. The Thermo Scientific™ Pierce™ qIP Protein Interaction Tluc Assay Reagents are only optimized for the Tluc Luciferase assay for protein interaction studies.

Assay Principle

A schematic of the qIP assay mechanism is shown in Figure 1. A protein of interest (labeled X) is tagged with an epitope tag and a second, potentially interacting protein (labeled Y) is fused to Tluc Luciferase. The two proteins are transiently expressed in mammalian cells following transfection. Cells are lysed and the epitope-tagged protein X is pulled down with anti-epitope agarose resin or magnetic beads. A luciferase assay is performed to quantitatively measure protein X-Y interactions without Western blotting. The light output is directly proportional to the amount of protein Y bound to protein X. The protein interaction is represented by the normalized signal-to-noise ratio. For the negative control, the protein Y-Tluc Luciferase fusion, together with epitope-tagged red fluorescence protein (RFP), is expressed separately.

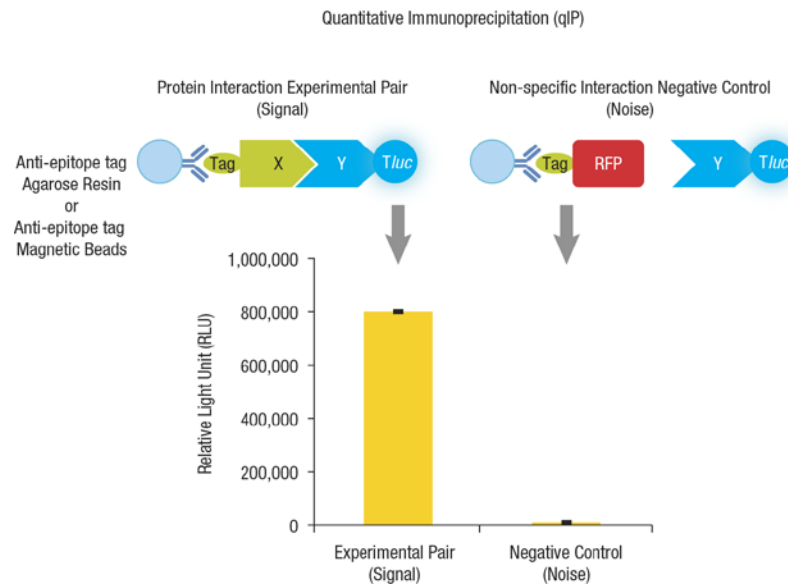


Figure 1. Schematic of qIP assay mechanism and resulting data.

Procedure Summary

Two plasmids, one encoding the epitope-tagged protein of interest (labeled X, Figure 2) and one encoding the potential interaction protein (labeled Y, Figure 2) fused to Tluc Luciferase, are transfected into mammalian cells. For the negative control, a plasmid for the protein Y-Tluc Luciferase fusion alone is separately transfected into the cells. After 24 hours, cells are collected, lysed and centrifuged to generate clear total lysate. The total lysates are incubated with anti-epitope agarose or magnetic beads for approximately 3 hours. The anti-epitope resin/beads are washed and resuspended in wash buffer. The resuspended resin slurry is transferred to 96-well plate (triplicates per sample). Luciferase substrate solution is added to each well, incubated for 10 minutes and relative light units (RLU) are measured with a luminometer.

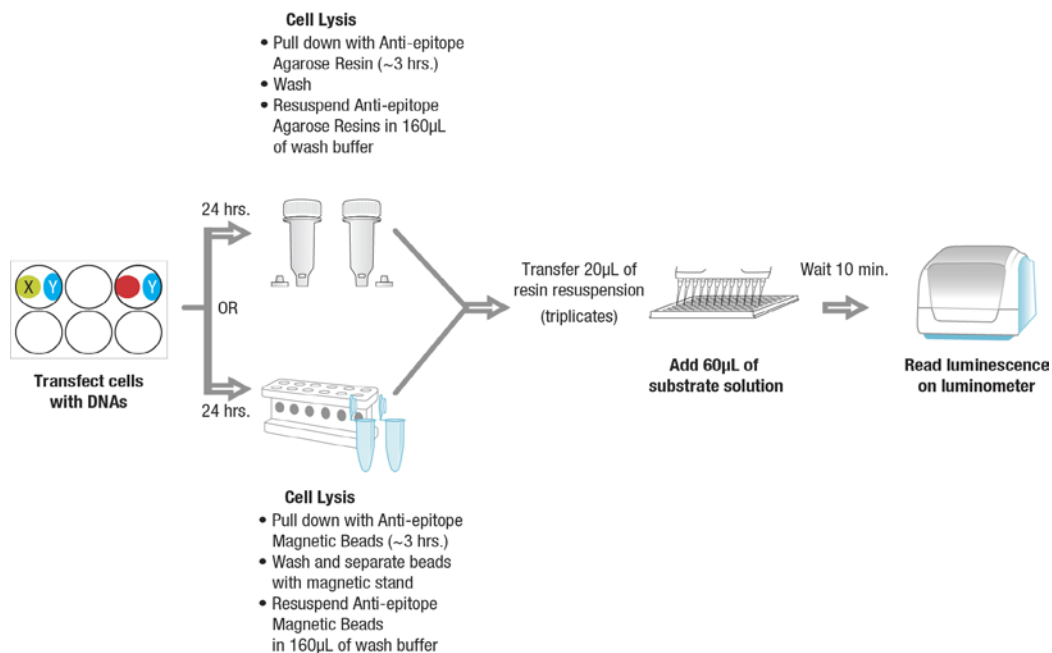


Figure 2. Schematic of qIP assay procedure.
Related Thermo Scientific Products

82013	Pierce qIP Protein Interaction Buffer L (1X) , 140mL, sufficient for 100 qIP reactions
82014	Pierce qIP Protein Interaction Buffer D (10X) , 50mL, sufficient for 100 qIP reactions
82017	pCMV MCS N-HA (N-terminus HA tag expression vector)
82018	pCMV MCS C-HA (C-terminus HA tag expression vector)
82019	pCMV MCS N-Myc (N-terminus Myc tag expression vector)
82020	pCMV MCS C-Myc (C-terminus Myc tag expression vector)
82023	pCMV MCS N-Tluc (N-terminus Tluc tag expression vector)
82024	pCMV MCS C-Tluc (C-terminus Tluc tag expression vector)
82025	pCMV RFP C-HA (C-terminus HA tag negative control vector)
82026	pCMV RFP C-Myc (C-terminus Myc tag negative control vector)
82028	pCMV BAD C-HA (C-terminus HA tag positive control vector)
82029	pCMV BAD C-Myc (C-terminus Myc tag positive control vector)
82031	pCMV Bcl-xL N-Tluc (N-terminus Tluc tag positive control vector)
82032	Pierce Agarose qIP Protein Interaction Kit, Tluc and HA Tags , 25 qIP reactions
82033	Pierce Agarose qIP Protein Interaction Kit, Tluc and Myc Tags , 25 qIP reactions
82035	Pierce Magnetic qIP Protein Interaction Kit, Tluc and HA Tags , 25 qIP reactions
82036	Pierce Magnetic qIP Protein Interaction Kit, Tluc and Myc Tags , 25 qIP reactions
R0533	TurboFect™ Transfection Reagent
78437	 Halt™ Protease Inhibitor Cocktail, EDTA-free
28374	Dulbecco's Phosphate-Buffered Saline (DPBS)
26181	Pierce Anti-HA Agarose, 2mL
88836	Pierce Anti-HA Magnetic Beads, 1mL
20168	Pierce Anti-c-Myc Agarose, 2mL
88842	Pierce Anti-c-Myc Magnetic Beads, 1mL
69705	Pierce Spin Columns – Screw Cap, 25 columns
5250030	Varioskan™ Flash with top reading
5250040	Varioskan Flash with top and bottom reading
5250500	Varioskan LumiSens Option, factory fitted (also enabling luminometric spectral scanning)
5250510	Dispenser option, with 1 mL syringe, factory fitted

General References

Vidal, M., Cusick, M.E. and Barabasi, A-L. (2011). Cell 144:986-998.

Muhammed, A., et al. (2007). Nature Biotechnology 27:1119-1126.

Stockwell, B. (2012). <http://blogs.nature.com/soapboxscience/2012/02/15/does-a-new-treatment-for-leukemia-herald-a-new-era-in-drug-discovery>

Chen J., et al. (2011). Mol Cancer Ther. 10:2340-9.

Limited Use Label License: Tluc Luciferases genes

(Thermo Scientific Product Nos. 82023, 82024, 82031, 82032, 82033, 82035, 82036)

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In addition, all buyers must either

a) for determinations of Tluc luciferase activity, use Thermo Scientific Pierce qIP protein Interaction Reagents (listed below)

Product Nos. 82013: Pierce qIP Protein Interaction Buffer L (1X), 140mL, sufficient for 100 qIP reactions

Product Nos. 82014: Pierce qIP Protein Interaction Buffer D (10X), 50mL, sufficient for 100 qIP reactions

Product Nos. 82015: Pierce qIP Protein Interaction Tluc Assay Reagents, 150 luciferase assay reactions

Product Nos. 82016: Pierce qIP Protein Interaction Tluc Assay Reagents, 1500 luciferase assay reactions

Product Nos. 82017: pCMV MCS N-HA, 10µg (0.5µg/µl)

Product Nos. 82018: pCMV MCS C-HA, 10µg (0.5µg/µl)

Product Nos. 82019: pCMV MCS N-Myc, 10µg (0.5µg/µl)

Product Nos. 82020: pCMV MCS C-Myc, 10µg (0.5µg/µl)

Product Nos. 82025: pCMV RFP C-HA, 10µg (0.5µg/µl)

Product Nos. 82026: pCMV RFP C-Myc, 10µg (0.5µg/µl)

Product Nos. 82028: pCMV BAD C-HA, 10µg (0.5µg/µl)

Product Nos. 82029: pCMV BAD C-Myc, 10µg (0.5µg/µl)

Product Nos. 82032: Pierce Agarose qIP Protein Interaction Kit, Tluc and HA Tags, 25 qIP reactions

Product Nos. 82033: Pierce Agarose qIP Protein Interaction Kit, Tluc and Myc Tags, 25 qIP reactions

Product Nos. 82035: Pierce Magnetic qIP Protein Interaction Kit, Tluc and HA Tags, 25 qIP reactions

Product Nos. 82036: Pierce Magnetic qIP Protein Interaction Kit, Tluc and Myc Tags, 25 qIP reactions

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