

## TurboLuc One-Step Glow Assay Kit

88263 88264

2553.0

Number	Description
88263	<b>TurboLuc One-Step Glow Assay Kit</b> , sufficient reagents to perform 100 assays for TurboLuc luciferase activity in cultured mammalian cells  <b>Kit Contents:</b> <b>TurboLuc One-Step Substrate (50X)</b> , 0.2mL <b>TurboLuc One-Step Assay Buffer</b> , 10mL
88264	<b>TurboLuc One-Step Glow Assay Kit</b> , sufficient reagents to perform 1,000 assays for TurboLuc luciferase activity in cultured mammalian cells  <b>Kit Contents:</b> <b>TurboLuc One-Step Substrate (50X)</b> , 1mL × 2 <b>TurboLuc One-Step Assay Buffer</b> , 100mL

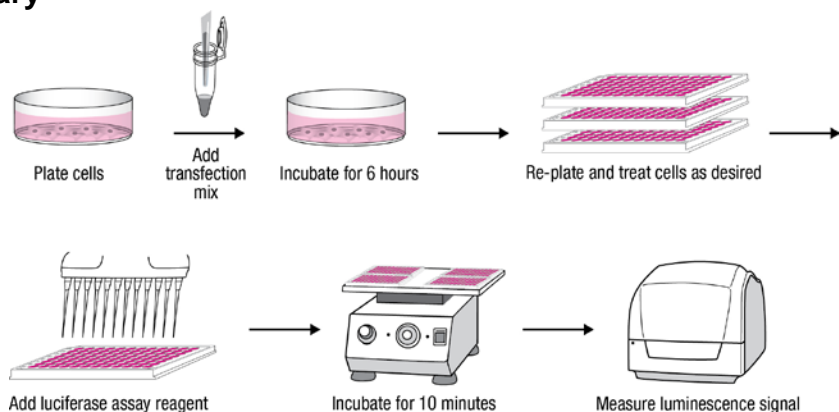
**Storage:** Upon receipt store at -80°C. Product shipped on dry ice.

## Introduction

The Thermo Scientific™ TurboLuc™ One-Step Glow Assay Kit provides a highly sensitive luciferase-based assay to measure promoter activity, study upstream cellular pathway activity, or determine the effect of activators or inhibitors. Thermo Scientific™ TurboLuc™16 (Tluc16) Luciferase and the Thermo Scientific™ TurboLuc™ One-Step Glow Assay reagents form a convenient, one-step homogeneous protocol that supports use in automated formats, including luminometers without injectors as well as high-throughput screening (HTS) applications.

Tluc16 is a novel 16kDa intracellular luciferase derived from the marine copepod *Metridia* luciferase family. The wild-type luciferase has been modified to reduce its size, increase brightness and express intracellularly. The Tluc16 gene was also developed using directed mutagenesis for optimal performance to suit HTS applications. Tluc16 is an ATP-independent enzyme that uses the substrate coelenterazine to produce intense blue bioluminescent light, enabling detection of very minute amounts of luciferase (signal). In addition, the Tluc16 luciferase expression vector contains the patented dual-destabilization technology that reduces accumulation of the Tluc16 mRNA and Tluc16 protein in cells, enhancing responsiveness to the Tluc16 gene and improving the sensitivity of the Tluc16 reporter system.

## Procedure Summary



## Important Product Information

- Store Thermo Scientific™ TurboLuc™ One-Step Substrate protected from light at -80°C.
- Store Thermo Scientific™ TurboLuc™ One-Step Assay Buffer at -80°C.
- TurboLuc One-Step Glow Assay working solution must be at room temperature (20-25°C) before use. The working solution loses ~25% activity over 6 hours at room temperature.
- To avoid cross-contamination, use a new disposable pipette tip for each transfer. If using a multi-channel pipette, always use a new disposable reagent reservoir.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different lots. Discard unused working solutions after assay completion. Do not combine remaining reagents.
- Individual components may contain corrosives and/or preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Follow proper disposal procedures.
- Dispense and equilibrate to room temperature only the reagent volumes needed for the number of plates being used.

## Additional Materials Required

- Reagents and equipment necessary to propagate mammalian cells in culture
- Reagents (e.g., Thermo Scientific™ TurboFect™ *in vitro* Transfection Reagent, Product No. R0533) and equipment necessary to transfect plasmid DNA into mammalian cells
- Laboratory platform shaker
- Pipettes and/or liquid handling equipment
- Luminometer or other luminescence-monitoring instrument
- White or black, opaque 96- or 384-well microplates
- Plasmid DNA containing the Tluc16 luciferase gene

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## Material Preparation (optional)

**Note:** Prepare the working solution 20 minutes before use.

Working solution	Dilute 50X TurboLuc One-Step Substrate 1:50 into TurboLuc One-Step Assay Buffer and mix well (e.g., for 100 reactions, add 200µL of 50X TurboLuc One-Step Substrate to 10mL of TurboLuc One-Step Assay Buffer). Prepare sufficient reagent to allow for dispensing errors and equipment void volumes. Use a volume of working solution equal to the sample volume present in each well of the test plate (e.g., if the sample volume is 100µL, then add 100µL of working solution).
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## Procedure for One-Step Glow Assay

### A. Cell Transfection

#### Day 1

1. Plate cells in a 6-well plate (1.5 million cells/well) or 10cm cell-culture dishes (4 million cells/dish). Scale up or down to suit other plate sizes; maintain the same ratios.

#### Day 2

1. Use a standard protocol to transfect mammalian cells with a Tluc16 luciferase plasmid construct containing a constitutive (e.g., EF1α, etc.) or an inducible promoter.
2. Incubate for 6 hours at 37°C. Trypsinize the cells. Re-suspend in complete media and re-plate into the desired plate(s) at the desired cell number. Recommended cell number is 20,000 cells/well in a 96-well plate.
3. Incubate cells at 37°C in 5% CO<sub>2</sub> in a cell culture incubator.

#### Day 3

1. Proceed with cell treatment and perform the luciferase assay as described below.

### B. TurboLuc One-Step Glow Assay

1. Remove the cell culture plate(s) from the incubator.
2. Allow the plate(s) to equilibrate to room temperature for 15 minutes.
3. Add an equal volume of working solution as the sample volume present in each well of the microplate.
4. Shake the plate on a plate shaker at medium speed for 10 minutes.
5. Read luminescence.

## Troubleshooting

Problem	Possible Cause	Solution
No or low signal	Low transfection efficiency	Optimize transfection conditions using a visual transfection control (e.g., a plasmid over-expressing a fluorescent protein)
		Verify plasmid DNA quality using restriction digestion and agarose gel electrophoresis (most high-quality plasmid DNA should be supercoiled)
		Use actively dividing, low-passage cells
		Use a different cell type
	No promoter induction	Incubate cells under promoter-specific inducing conditions
		Incubate the cells for a longer time after treatment
		Change growth conditions to improve expression
		Use a different promoter or cell type
	TurboLuc One-Step Substrate auto-oxidized	Protect substrate from light and air and store at -80°C
		Prepare working solution immediately before use and protect from light
	Wrong substrate used	Use only substrate supplied with the kit. Coelenterazine from related luciferase kits (e.g., <i>Renilla</i> luciferase) will provide suboptimal performance
	Low luciferase expression	Use a different promoter or growth conditions to improve expression
		Adjust instrument parameters to capture more signal (instrument-dependent)
		Plate larger number of cells
Signal is high or the signal quickly decays	High luciferase expression	Reduce incubation time before collecting samples
		Adjust instrument parameters to capture less signal (instrument-dependent)
		Decrease the amount of plasmid transfected into cells or decrease cell number
	Control sample contaminated	Change pipette tips after each well

## Related Thermo Scientific Products

<b>R0533</b>	<b>TurboFect <i>in vitro</i> Transfection Reagent</b>
<b>88951-2</b>	<b>alamarBlue™ Cell Viability Assay Reagent</b>
<b>88853-4</b>	<b>Pierce™ LDH Cytotoxicity Assay Kit</b>
<b>28374</b>	<b>Modified Dulbecco's PBS Packs</b>
<b>88231</b>	<b>pMCS Tluc16-DD</b>
<b>88232</b>	<b>pMCS minP-Tluc16-DD</b>
<b>88235</b>	<b>pMCS Tluc16</b>
<b>88236</b>	<b>pMCS minP-Tluc16</b>
<b>88241</b>	<b>pEF1α Tluc16</b>
<b>88246</b>	<b>pNFκB Tluc16-DD</b>
<b>88247</b>	<b>pCRE Tluc16-DD</b>
<b>88248</b>	<b>pMCS Tluc16-DD Hygro</b>
<b>88249</b>	<b>pMCS minP-Tluc16-DD Hygro</b>
<b>88255</b>	<b>pMCS Tluc16 Hygro</b>
<b>88256</b>	<b>pMCS minP-Tluc16 Hygro</b>

## General Reference

Voon, Dominic C., *et al.* (2005). Use of mRNA- and protein-destabilizing elements to develop a highly responsive reporter system. *Nucleic Acids Research* 33(3)e27.

**Limited Use Label License: Thermo Scientific TurboLuc™ (Tluc) Luciferase Gene Products for qIP Protein Interaction (Thermo Scientific Product Nos. 82017, 82018, 82019, 82020, 82023, 82024, 82025, 82026, 82028, 82029, 82031) and One-Step Glow Assay (Thermo Scientific Product Nos. 88231, 88232, 88235, 88236, 88241, 88246, 88247, 88248, 88249, 88255, 88256)**

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In addition, the buyer shall:

(a) only use the Thermo Scientific TurboLuc™ (Tluc) Luciferase gene products listed in Group A in the table below with the Thermo Scientific Pierce qIP Protein Interaction reagents listed in Group B in the table below, and vice-versa, for determinations of Tluc luciferase activity:

<b>Group A: TurboLuc™ Luciferase gene products for qIP</b>
Product No. 82017: pCMV MCS N-HA
Product No. 82018: pCMV MCS C-HA
Product No. 82019: pCMV MCS N-Myc
Product No. 82020: pCMV MCS C-Myc
Product No. 82023: pCMV MCS N-Tluc
Product No. 82024: pCMV MCS C-Tluc
Product No. 82025: pCMV RFP C-HA
Product No. 82026: pCMV RFP C-Myc
Product No. 82028: pCMV BAD C-HA
Product No. 82029: pCMV BAD C-Myc
Product No. 82031: pCMV Bcl-xL N-Tluc
<b>Group B: qIP Protein Interaction reagents</b>
Product No. 82013: Pierce™ qIP Protein Interaction Buffer L (1X)
Product No. 82014: Pierce™ qIP Protein Interaction Buffer D (10X)
Product No. 82015: Pierce™ qIP Protein Interaction Tluc Assay Reagents
Product No. 82016: Pierce™ qIP Protein Interaction Tluc Assay Reagents
Product No. 82032: Pierce™ Agarose qIP Protein Interaction Kit, Tluc and HA Tags
Product No. 82033: Pierce™ Agarose qIP Protein Interaction Kit, Tluc and Myc Tags
Product No. 82035: Pierce™ Magnetic qIP Protein Interaction Kit, Tluc and HA Tag
Product No. 82036: Pierce™ Magnetic qIP Protein Interaction Kit, Tluc and Myc Tag

(b) only use the Thermo Scientific TurboLuc™ Luciferase One-Step Glow Assay gene products listed in Group C in the table below with the TurboLuc™ Luciferase One-Step Glow Assay reagents listed in Group D in the table below, and vice-versa, for determinations of Tluc luciferase activity:

<b>Group C: TurboLuc™ Luciferase One-Step Glow Assay gene products</b>
Product No. 88231: pMCS Tluc16-DD
Product No. 88232: pMCS minP-Tluc16-DD
Product No. 88235: pMCS Tluc16
Product No. 88236: pMCS minP-Tluc16
Product No. 88241: pEF1α Tluc16-DD
Product No. 88246: pNFkB Tluc16-DD

Product No. 88247: pCRE Tluc16-DD
Product No. 88248: pMCS Tluc16-DD Hygro
Product No. 88249: pMCS minP-Tluc16-DD Hygro
Product No. 88255: pMCS Tluc16 Hygro
Product No. 88256: pMCS minP-Tluc16 Hygro
<b>Group D: TurboLuc™ Luciferase One-Step Glow Assay reagents</b>
Product No. 88263: TurboLuc™ Luciferase One-Step Glow Assay Kit
Product No. 88264: TurboLuc™ Luciferase One-Step Glow Assay Kit

or

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