TITANIUM™ ONE-STEP LUCIFERASE ASSAY KIT

REF : CC5002

Ver1.1

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RIUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Product Description:

The Titanium™ One-Step Luciferase Assay Kit is a highly sensitive, robust, homogeneous reagent for the detection of firefly luciferase reporter gene expression in the ADCC/ADCP or other Reporter Bioassays.

The luciferase reporter assay is commonly used as a tool to study gene expression at the transcriptional level. It is widely used because it is convenient, relatively inexpensive, and gives quantitative measurements instantaneously. It has broad applications across various fields of cell and molecular biology-wherever you want to measure or track expression of a cloned gene. Luciferase is a popular choice as a reporter for these applications because functional enzyme is created immediately upon translation, and the assay is rapid, reliable and easy to perform. Furthermore, analysis using luciferase as the genetic reporter is well suited to laboratory automation and high-throughput applications. For these reasons, luciferase is widely used in the biotechnology and pharmaceutical industries.

The Titanium™ One-Step Luciferase Assay Kit provides a highly sensitive, robust, homogeneous reagent for the detection of firefly luciferase reporter gene expression in the ADCC, ADCP or other reporter bioassays. The assay kit consists of D-luciferin, which is a substrate of firefly luciferase. Firefly luciferase is a 61kDa monomer that catalyzes the mono-oxygenation of D-luciferin (Figure 1). D-luciferin is a relatively stable molecule found only in luminous beetles (which includes fireflies). The enzyme catalyzes luciferin oxidation using ATP.Mg2+ as a co-substrate (Figure 1).

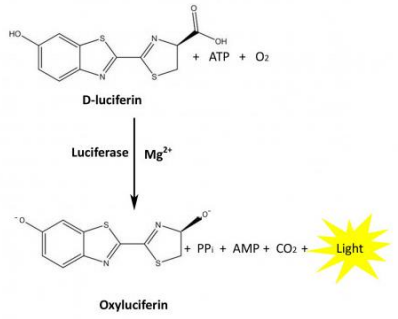


Fig 1. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg2+, ATP and molecular oxygen.

Product Size:
Each kit contains sufficient reagents to perform 660 assays of 75 ul each.

Product Components:
Titanium™ One-Step Luciferase Assay Kit consists of:

Details	Size
Titanium™ Luciferase Assay Buffer	50 ml
Titanium™ Luciferase Assay Substrate (lyophilized)	1 vial

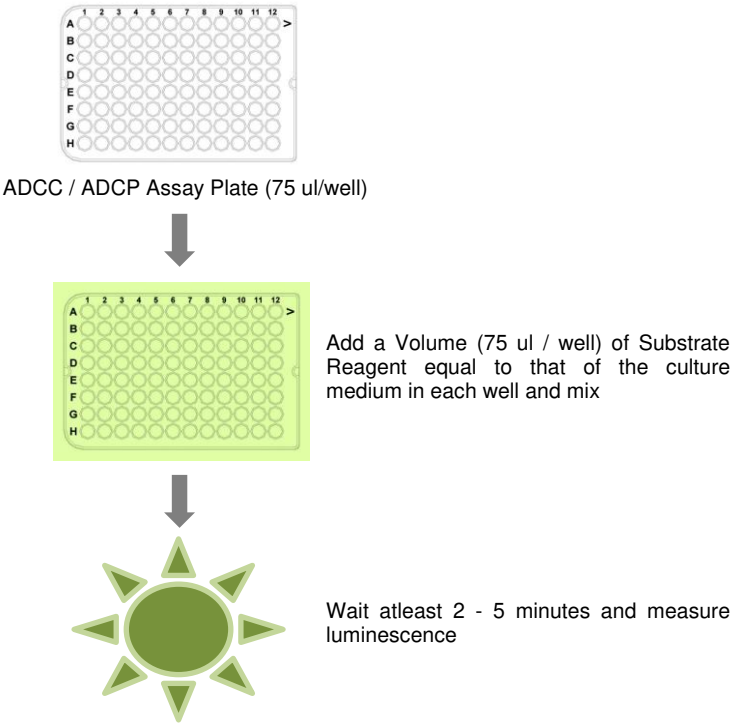
Storage Conditions:
The Titanium™ One-Step Luciferase Assay Kit is shipped on dry ice and must be stored at -70°C upon receipt. Reconstituted Luciferase Assay Substrate Reagent (Luciferase Assay Substrate + Luciferase Assay Buffer) should be stored in aliquots at -70°C for up to 6 months. Avoid repeated freeze-thaw cycles.

Product Characteristic:
Titanium™ One-Step Luciferase Assay Kit has the following advantages:

- Fast: luciferase test was completed in 10-15min ;
- Convenient: the mixture of lyophilized powder and buffer can be used simply, and the sample detection procedure is simple ;
- High sensitivity: the assay sensitivity of luciferase is 10-20 mol;
- Ultra-high luminescent intensity: Stronger light output, producing light intensity 10 times higher than other luciferase detection reagents.

Titanium™ One-Step Luciferase Assay Kit is suitable for routine detection and lot release detection of ADCC or ADCP activity. At the same time, the kit can also be used to detect the bioactivity of small molecule compounds and recombinant proteins, such as IFN, EPO, FSH, TSH, GH, GLP-1, PTH etc.

Assay Procedure:
The assay may be set-up as per the flow diagram and details below.



A. Substrate Reagent Preparation
For optimal performance, use reconstituted Titanium™ Substrate Reagent on the day of preparation. However, once reconstituted, Titanium™ Substrate Reagent can be stored at -70°C for up to 6 months.

1. Thaw the Titanium™ Luciferase Assay Buffer in a refrigerator overnight or in a room temperature water bath on the day of the assay;
2. Equilibrate the Titanium™ Assay Buffer to ambient temperature, protected from light;
3. Transfer the 50 ml of Titanium™ Luciferase Assay Buffer into the amber bottle containing the Titanium™ Luciferase Assay Substrate, and mix by inversion until the Substrate is thoroughly dissolved;
4. Equilibrate the reconstituted Titanium™ Reagent to ambient temperature before adding to assay plates.

Note:
1) Dispense the reconstituted Reagent into 10 ml aliquots and store at -70°C for up to six months. Avoid repeated freeze-thaw cycles;
2) On the day of the assay, thaw the appropriate amount of reconstituted Reagent in a room temperature water bath for 4-6 hours before use.

B. Preparing Cell Lysates
1. Equilibrate to ambient temperature
2. Remove assay plates from the 37°C incubator and equilibrate to ambient temperature (22-25°C) on the bench for 5-15 minutes.
3. *Note: 96-well Microplate needs to be matched with luminometer, such as white 96-well Microplate.*
4. Adding Titanium™ Substrate Reagent: Using a manual multichannel pipette, add a volume of Titanium™ Substrate Reagent equal to the volume of cell lysates (e.g. 100 µl per well of a 96-well plate). Avoid creating any bubbles.
5. *Note: Titanium™ Substrate Reagent should be at ambient temperature when added.*
6. Incubation: Wait at least 2-5 minutes to allow cell lysis, then measure luminescence in a luminometer.
7. Measure luminescence in a luminometer immediately after Titanium™ Substrate Reagent is added.

Data Analysis:

1. Calculate the average value of background value, samples value and negative control value, and analyze the data based on the results.
2. Calculate Fold of Induction = RLU (induced-background) / RLU (no antibody control-background).
   Note: When calculating Fold of Induction, if the sample RLUs is equal to or greater than 100 times higher than the plate background RLU, there is no need to subtract plate background from sample RLU
3. Graph data as RLU versus Log10 [antibody] and Fold of Induction versus Log10 [antibody]. Fit curves and determine EC50 of antibody response using appropriate curve fitting software.

Notes to the Procedure:

- Applicable cell type: This kit is also suitable for the detection of luciferase in suspension cells and adherent cells without removing the culture medium.
- Applicable medium type: This kit is compatible with RPMI1640, DMEM/F12, MEM and DMEM high glucose medium (containing 0-10% serum); if other medium or serum concentration is used, validation of the same is recommended.
- Influencing factors: The test results will be affected by various factors such as phenol red, organic solvents and detection temperature. The presence of phenol red will reduce the luminescence intensity. It is recommended to use the phenol red-free medium provided by KRISHGEN BIOSYSTEMS for cell culture.
- This kit is compatible with DMSO, isopropanol and ethanol. All reagents and cells need to be equilibrated to ambient temperature for testing.
- Experimental operation: The signal half-life of this kit is 10 min, so it is necessary to perform fluorescence signal detection after adding the substrate reagent for 2-5 min.

Troubleshooting:

No Signal or Low Signal

There may be several reasons for no signal or low signal: D-Luciferin auto-oxidized or Low luciferase expression and degradation of luciferase. The corresponding solutions or precautions are as follows:

D-Luciferin auto-oxidized	- Protect substrate from light and air; - Dispense the reconstituted Titanium™ Reagent into 8ml aliquots and store at -70°C; - Avoid repeated freeze-thaw cycles;
Low Luciferase Expression	- Use a different promoter or growth conditions to improve expression; - Scale-up the volume of sample and reagent per well;

References:

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