Luciferase Assay Systems

INSTRUCTIONS FOR USE OF PRODUCTS E1483, E1500, E1501, E1531, E4030, E4530 AND E4550.

Quick PROTOCOL

Reagent and Cell Extract Preparation

1. For kits E1500, E1501, E4030, E4530 and E4550, prepare **Luciferase Assay Reagent** (**LAR**) by adding **Luciferase Assay Buffer** (10ml for E152A and 100ml for E152B) to the vial of lyophilized **Luciferase Assay Substrate**. Dispense into working aliquots and store unused **LAR** at –20°C or –70°C. Before each use of the system, allow **LAR** to equilibrate to room temperature. (Do not thaw **LAR** at temperatures above 25°C.)
2. Prepare 1X lysis reagent by adding 4 volumes of water to 1 volume of 5X lysis reagent (**Cell Culture Lysis Reagent** [**CCLR**], Cat.# E1531; **Reporter Lysis Buffer** [**RLB**], Cat.# E3971; or **Passive Lysis Buffer** [**PLB**], Cat.# E1941).

Preparation of Mammalian Cell Lysate

1. Remove growth medium from cultured cells.
2. Rinse cells in 1X PBS. Do not dislodge cells. Remove as much of the final wash as possible.
3. Dispense a minimal volume of 1X lysis reagent (**CCLR**, **RLB** or **PLB**) into each culture vessel (e.g., 400µl/60mm culture dish, 900µl/100mm culture dish or 20µl/well for a 96 well plate).
4. For culture dishes, scrape attached cells from the dish, and transfer the cells and solution to a microcentrifuge tube. Pellet debris by brief centrifugation, and transfer the supernatant to a new tube.
5. Mix 20µl of cell lysate with 100µl of Luciferase Assay Reagent and measure the light produced.

Preparation of Plant Tissue Lysate

1. Quick-freeze the tissue in liquid nitrogen, grind the frozen tissue to a powder and resuspend in room temperature 1X lysis reagent with homogenization.
2. Pellet debris by brief centrifugation and transfer supernatant to a new tube.
3. Mix 20µl of cell lysate with 100µl of Luciferase Assay Reagent and measure the light produced.

Preparation of Bacterial Cell Lysate

1. Mix 40µl of untransformed bacteria (“carrier cells”) with 50µl of transformed culture.
2. Add 10µl of 1M K_2HPO_4 (pH 7.8) and 20mM EDTA.
3. Quick-freeze on dry ice, and then equilibrate to room temperature by placing the tube in room temperature water.
4. Add 300µl freshly prepared lysis mix (1 volume of freshly prepared lysozyme and 2 volumes of **2X CCLR** with 5mg/ml BSA). Mix and incubate for 10 minutes at room temperature.
5. Mix 20µl of cell lysate with 100µl of Luciferase Assay Reagent and measure the light produced.

See additional protocol information in Technical Bulletin #TB281, available online at:
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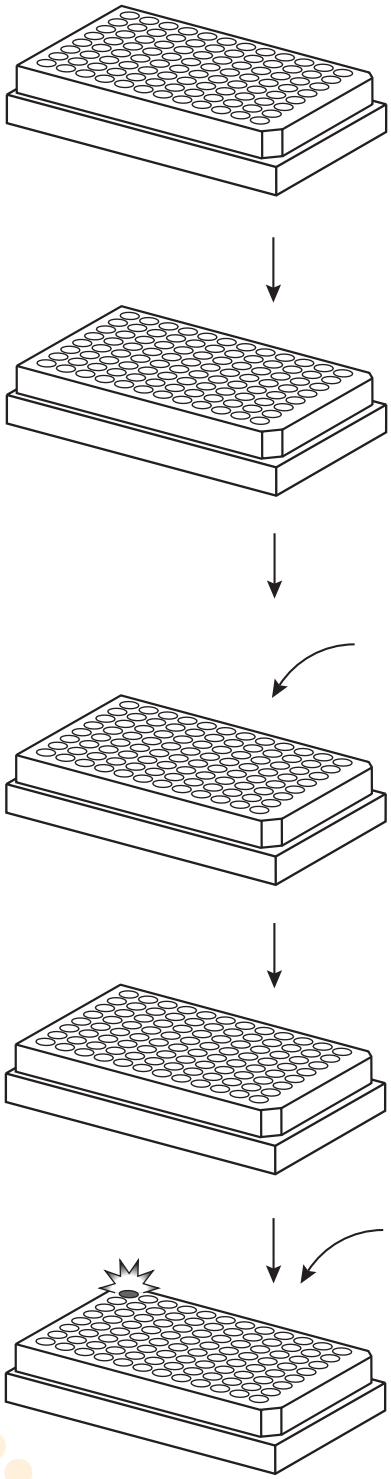
Quick PROTOCOL

Standard 96 Well Plate Assay



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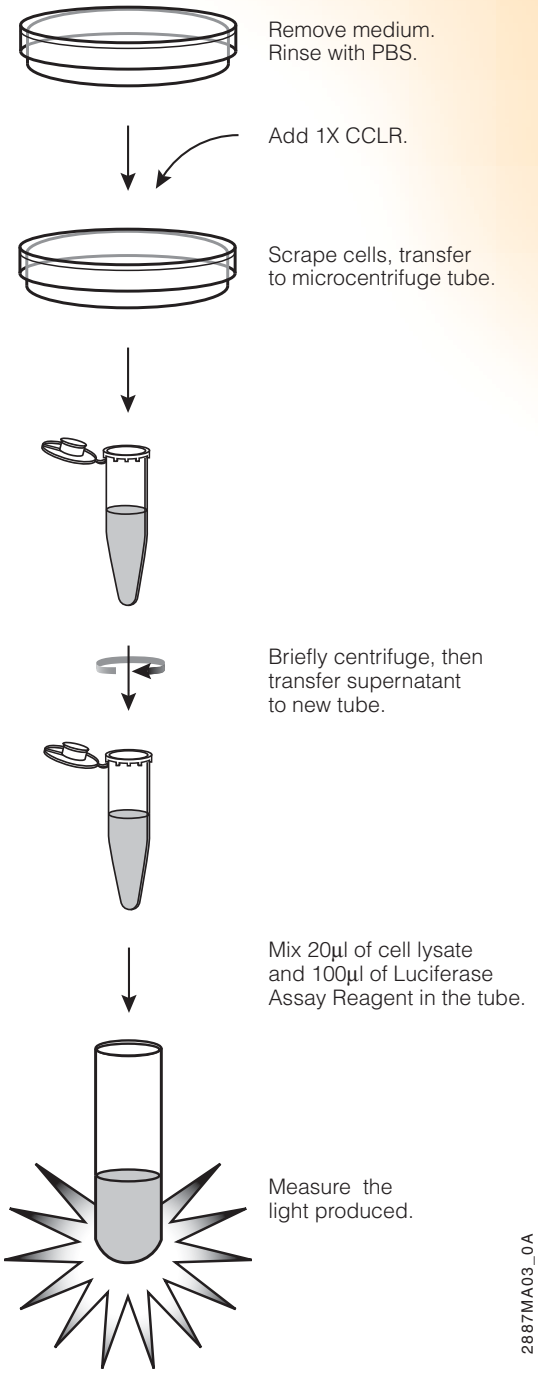
Standard Culture Dish Assay



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