QUICK START GUIDE

Twinlite

Firefly & Renilla Luciferase
Reporter Gene Assay System

A RESEARCH PRODUCT FOR RESEARCH PURPOSES ONLY

Instructions for use of product **6066706** (100 assay kit, 96-well format)

Reagent Preparation

1. Allow all reagents to reach room temperature (20 – 22°C).
2. **Passive Lysis Solution:** PLS is a ready to use reagent.
3. **firelite plus reagent:**
    - Reconstitute the vial *fireflite plus Lyophilized Substrate* by adding the full contents of the 12 mL bottle sensilite Reconstitution Buffer.
    - Mix the contents of the vial gently by inversion and leave for 5 minutes.

Unused reagent can be stored at –20°C (≤ 2 months) or –80°C (≤ 2 year).

Cell Lysate Preparation

1. Remove cell growth medium from the cell layer.
2. Wash the cells with a sufficient amount of PBS at room temperature. Swirl briefly to remove loose cells and residual growth medium. Remove the wash solution as much as possible.
3. Add to the cell layer the recommended volume of PLS according to the table below.

Plate type	PLS/well
6 well	500µL
12 well	250µL
24 well	100µL
48 well	65µL
96 well	20µL

4. Place the plate on an orbital shaker or on a rocking platform so that the PLS covers the cell layer evenly for optimal lysis. Shake the plate for 15 minutes at room temperature.

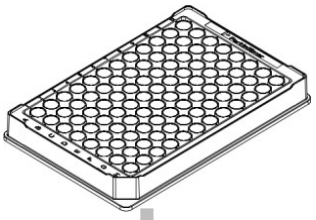
5. The cell lysate can now be used in the twinlite assay. If the lysate is not needed the same day, store at –20°C (≤ 2 months) or –80°C (≤ 1 year). When the cells are cultured in an opaque 96-well plate, then the assay can be performed directly in the same plate without lysate transfer.

Measuring firefly luciferase luminescence, sensilite assay

1. Set the luminometer injector 1 and 2 to dispense 100 µL firelite plus and renilite plus reagent.
2. Set a count delay of 2 seconds between the reagent injection and measuring luminescence. Set the luminescence read time between 5 to 10 seconds.
3. Fill and rinse the designated injectors of the luminometer with the prepared reagent.
4. Load the microplate containing the samples (20µL/well) in the luminometer, dark adapt for a few minutes to decrease plate phosphorescence (to lower plate background levels) and start the measurement.

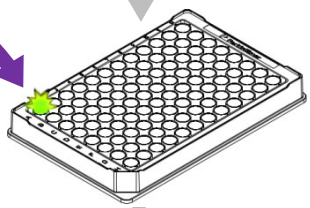
Assay using a 96-well plate (opaque)

96-well plate with 20 µL **PLS** lysate/well.



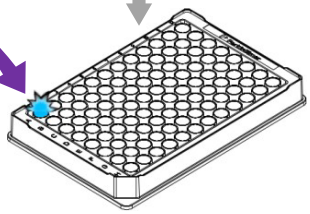
Inject 100 µL of **firelite plus**

Measure firefly luciferase luminescence for 5 – 10 seconds after a 2 seconds count delay.



Inject 100 µL of **renilite plus**

Measure firefly luciferase luminescence for 5 – 10 seconds after a 2 seconds count delay.



Repeat this cycle for the other samples in the plate.

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