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Instructions for use of product 6066726 (100 assay kit; 96-well format)

Reagent Preparation

- 1. Allow all reagents to reach room temperature $(20 22^{\circ}C)$.
- 2. Passive Lysis Solution: PLS is a ready to use reagent.
- 3. sensilite reagent:
 - Reconstitute the vial sensilite 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.

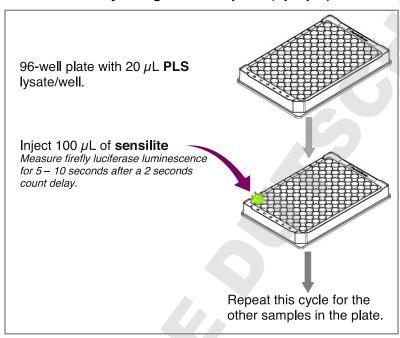
Plate type	PLS/well
6 well	500 μL
12 well	250 μL
24 well	100 <i>μ</i> L
48 well	65 μL
96 well	20 μL

- 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 sensilite 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 to dispense 100 μ L **sensilite** 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.
- 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)





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