



Enhanced Flash Luminescence -  
Firefly Luciferase Reporter Gene Assay System



## QUICK START GUIDE

A RESEARCH PRODUCT FOR RESEARCH PURPOSES ONLY

Instructions for use of product 6066726 (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. **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.

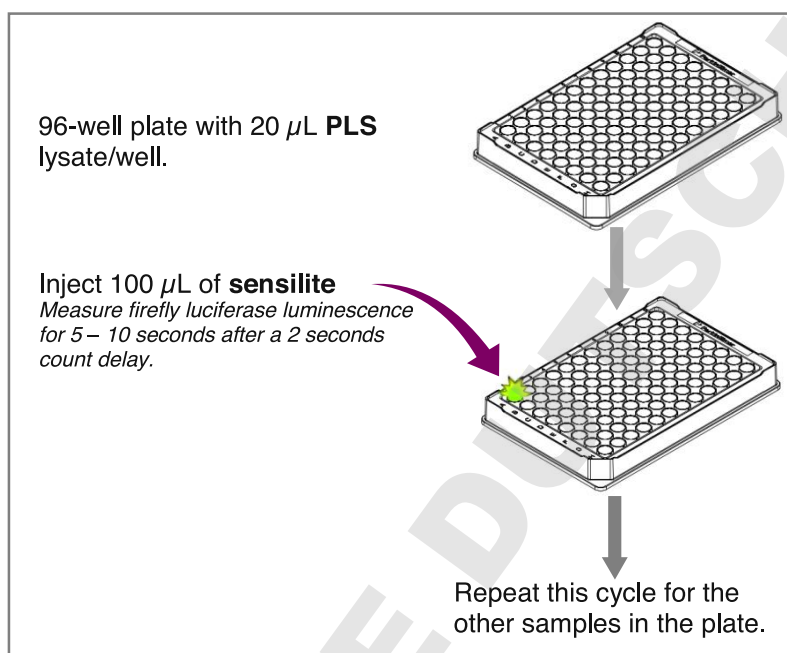
Plate type	PLS/well
6 well	500 $\mu$ L
12 well	250 $\mu$ L
24 well	100 $\mu$ L
48 well	65 $\mu$ L
96 well	20 $\mu$ L

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  $\mu\text{L}$  **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.
4. Load the microplate containing the samples (20 $\mu\text{L}$ /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)



For a complete listing of our global offices visit [www.perkinelmer.com](http://www.perkinelmer.com)

#### PerkinElmer, Inc.

940 Winter Street  
Waltham, MA 02451 USA  
Phone: (800) 762-4000 or  
(+1) 203-9254602  
[www.perkinelmer.com](http://www.perkinelmer.com)