

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

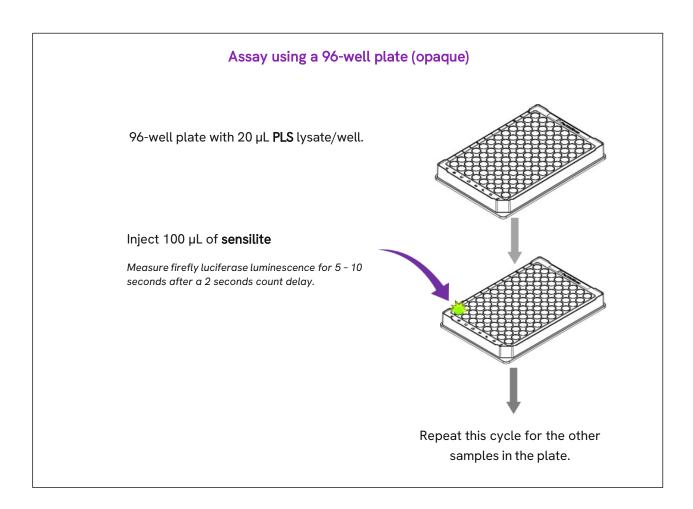
- 1. Remove cell growth medium from the cell layer.
- 2. Wash the cells with a sufficient amount of PBS at room temperature. Swirl briefly to remove loose cells and residual growth medium. Remove the wash solution as much as possible.
- 3. Add to the cell layer the recommended volume of PLS according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

6 well	500μL
Plate type	PLS/well
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 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.
- 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.



This product and/or its use is covered by the following patents and corresponding patent applications worldwide, owned by Revvity Health Sciences B.V.: US Patent No. 8,512,968; EP Patent No. EP2222870B1; China Patent No. CN101889095B; and Australia Patent No. AU2008319571B2.



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