

## Kit Contents and Handling

Component	Catalog no.	Amount	Storage	Handling
BacMam LRRK2-GFP Reagent (WT or G2019S)	A14687 or A14688	5 × 1 mL	4°C	DO NOT FREEZE Use sterile technique Protect from light
Tb-anti-LRRK2 pSer935 Antibody	A14689	5 µg	-20°C	Protect from light
LanthaScreen® 6X Cellular Assay Lysis Buffer	A12891	6 mL	4°C	On day of assay, add protease inhibitor*, phosphatase inhibitor*, and antibody
Instrument Control Terbium TR-FRET Kit	A14138	1 kit	4°C	Protect from light

\*See **Materials Required but Not Provided** on page 2.

**For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.**

## Overview

The **LanthaScreen® LRRK2 [pSer935] Cellular Assay Kit** combines the flexibility of BacMam gene delivery system with the robustness and power of the LanthaScreen® TR-FRET technology. It allows researchers to interrogate phosphorylation at serine 935 on Leucine-Rich Repeat Kinase-2 (LRRK2) protein in a variety of cell backgrounds to identify LRRK2 kinase inhibitors in a high throughput format. This kit provides enough material to perform the assay in a 384-well plate; larger sizes of all reagents are available through our catalog.

Figure 1 Assay Workflow. Day 1: Cells are transduced with BacMam reagent. Day 2: Cells are harvested and transferred to assay plate. Day 3: Following the treatment with or without LRRK2 inhibitor, cells are lysed in the presence of a Tb-labeled anti-LRRK2 pSer935 antibody and the levels of phosphorylation are measured using a Tb TR-FRET-compatible plate reader.

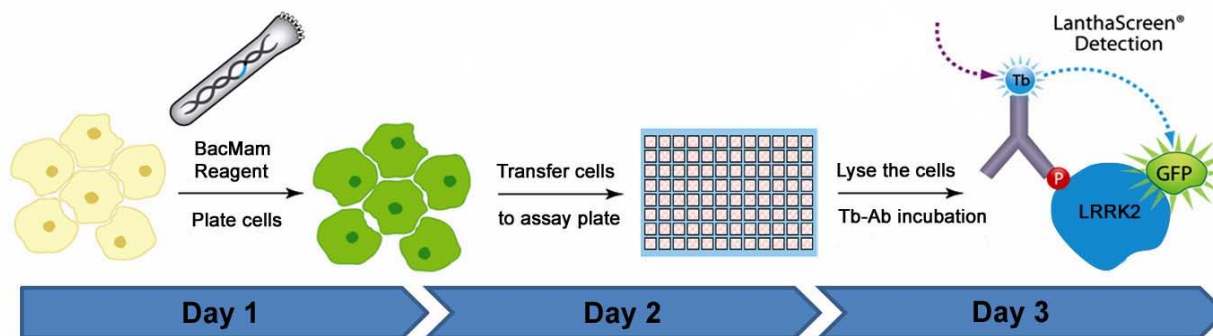
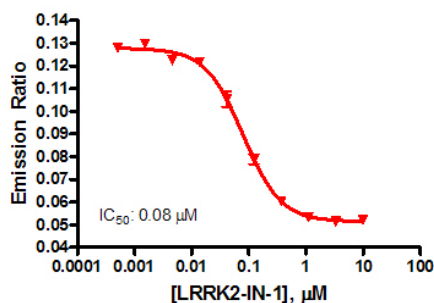


Figure 2 Representative data collected using U-2 OS cells transduced with BacMam LRRK2 G2019S.



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For information on frequently asked questions regarding the LanthaScreen® technology, go to [www.lifetechnologies.com/lanthascreen](http://www.lifetechnologies.com/lanthascreen)

## Before Starting

### Materials Required but Not Provided

Materials	Recommended Source	Part no.
<p><b>Fluorescence plate reader</b> with Terbium (Tb) TR-FRET (LanthaScreen®) capability.</p> <p>Prior to setting up an assay, we strongly recommend checking your plate reader setup for Tb-based TR-FRET (LanthaScreen®) detection using the Instrument Controls included with this kit.</p> <p>Tb-based TR-FRET (LanthaScreen®) technology requires specific instrument settings that are critical to experimental success. Also note that for this assay we do not recommend using monochromator-based instruments as the sensitivity of these instruments is generally not sufficient to detect the TR-FRET signal.</p>	<p>For instrument set-up guidance, refer to <a href="http://www.lifetechnologies.com/instrumentsetup">www.lifetechnologies.com/instrumentsetup</a>.</p> <p>For technical assistance, contact Drug Discovery Technical Support at <a href="mailto:drugdiscoverytech@invitrogen.com">drugdiscoverytech@invitrogen.com</a> or 760-603-7200, extension 40266).</p>	
<p><b>Cell Line of Interest</b> (visit <a href="http://www.lifetechnologies.com/bacmam">www.lifetechnologies.com/bacmam</a> for more information regarding BacMam compatible cell types; we have used this assay successfully with U-2 OS, HEK293T and SH-SY5Y cells)</p>	Various	Various
<p><b>BacMam Enhancer Solution (1000X)</b> (Do not use this solution if using U-2 OS cells)</p>	Life Technologies	PV5835
<p><b>Protease Inhibitor Cocktail</b></p>	Sigma	P8340
<p><b>Phosphatase Inhibitor Cocktail</b></p>	Sigma	P0044
<p><b>Assay Plate</b></p> <ul style="list-style-type: none"> <li>for TR-FRET, use white tissue culture-treated 384-well plates</li> <li>for visualization of LRRK2-GFP, use clear-bottom, tissue culture-treated plates</li> </ul>	<p>Corning</p> <p>Corning</p>	<p>3570</p> <p>3712</p>

## Assay Protocol

### Day 1: BacMam LRRK2-GFP Transduction

The first critical experiment is a titration of the BacMam virus in your cell line of interest. For easy-to-transduce cells, such as U-2 OS, HEK293, HeLa, and human mammary epithelial cells, we recommend following the standard transduction protocol. For more information, also see the protocol described for BacMam GFP control (Cat. no. B10383) available at [www.lifetechnologies.com](http://www.lifetechnologies.com).

**Note:** Expression levels of GFP Control and LRRK2-GFP do not correlate; therefore, optimal concentration of the GFP control virus may not apply to the BacMam LRRK2-GFP virus. To determine the optimal concentration of BacMam LRRK2-GFP reagents for your cell line of interest, you must perform a virus titration experiment.

We recommend testing a range of BacMam reagent dilutions (v/v) to determine the optimal percentage of virus for your transduction in the presence or absence of 0.5X BacMam Enhancer Solution (Cat. no. PV5835). As a starting point, we recommend using 50%, 30%, 20%, 10%, and 2% (v/v) of BacMam reagent for initial optimization.

- Harvest and resuspend cells in growth medium. Add BacMam LRRK2-GFP to cell suspension at the optimal concentration. For U-2 OS we recommend 20% (v/v). Mix gently by inversion. (For some cell types except U-2 OS, 0.5X BacMam Enhancer solution can be added at this step to increase expression level.) Transfer cells/BacMam reagent mixture to appropriate cell-culture plates (such as 6-well plates). Incubate plates in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub> for 20–24 hours.

**For SH-SY5Y cells and other difficult-to-transduce cells, such as human astrocytes and primary neurons, we recommend the following transduction protocol.**

- Start with SH-SY5Y cells already plated in a 6-well plate at 1.5 to 2 million cells/well in growth medium. Remove growth medium and wash cells once with PBS with Ca<sup>2+</sup> and Mg<sup>2+</sup> (Cat. no. 14040117). Add 1.5 mL of PBS with Ca<sup>2+</sup> and Mg<sup>2+</sup> to each well, and then add 0.5 mL of BacMam LRRK2-GFP reagent to 25% (v/v) final. Incubate the plate at room temperature in the dark with gentle rocking for 3–4 hours. Remove virus/PBS solution and add 2 mL/well of growth medium containing 0.3X BacMam Enhancer Solution. Incubate plate in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub> for 20–24 hours.

**Note:** Growth medium for SH-SY5Y consists of DMEM/F12 (Cat. no. 10565) containing 10% dialyzed FBS (Cat. no. 26400) and 1X penicillin/streptomycin (Cat. no. 15140).

### ***Day 2: Cell Plating onto a 384-well assay Plate***

1. After transduction, harvest and resuspended cells in Assay Medium at the following density (U-2 OS:  $0.5 \times 10^6$  cells/mL; HEK293:  $0.75 \times 10^6$  cells/mL; SH-SY5Y:  $1 \times 10^6$  cells/mL). Plate cells onto a 384-well assay plate (Corning, Cat. no. 3570) in 20  $\mu$ L/well volume.

*Optional:* Plate cells into wells of a clear bottom plate (Corning, Cat. no. 3712) for GFP visualization.

**Note:** Assay Medium consists of Opti-MEM I (Cat. no. 11058021) with 0.1% dialyzed FBS (Cat. no. 26400), 0.1 mM NEAA (Cat. no. 11140-050), 1 mM Sodium Pyruvate (Cat. no. 11360-070), and 100 U/mL Penicillin/100  $\mu$ g/mL Streptomycin (Cat. no. 15140-122)

2. Centrifuge plate briefly at  $300 \times g$ . Incubate plates in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub> for 20–24 hours.

### ***Day 3: Inhibitor Treatment, Cell Lysis and TR-FRET Detection***

1. Add 5  $\mu$ L/well of compounds or DMSO at 5X concentration in Assay Medium. Centrifuge plate briefly at  $300 \times g$ . Incubate plates in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub> for 60–90 minutes.
2. Prepare Complete 6X Lysis Buffer by adding protease inhibitor (Sigma, Cat. no. P8340) and phosphatase inhibitor (Sigma, Cat. no. P0044) cocktails to LanthaScreen® 6X Lysis Buffer (Cat. no. A12891), at a 1:33 dilution of 100X stock (e.g., 30  $\mu$ L of 100X stock inhibitors per 1,000  $\mu$ L of LanthaScreen® 6X Lysis Buffer) and add LanthaScreen® Tb-anti-LRRK2 [pSer935] Antibody to the 6X Lysis Buffer at a concentration of 12 nM. Mix gently by inversion. Store on ice until use.
3. Add 5  $\mu$ L/well of Complete LanthaScreen® 6X Lysis Buffer. Centrifuge plate briefly at  $300 \times g$ . Incubate at room temperature in the dark for 2–3 hours.
4. Read plate using instrument capable of detecting TR-FRET. See below for details on terbium TR-FRET detection or call our technical support team for assistance.

### ***Testing Terbium TR-FRET Detection Using the Instrument Controls***

To test your instrument set-up for performing terbium-based TR-FRET cellular assays:

1. Add 60  $\mu$ L/well of HIGH Control to empty assay plate wells for 96-well format (or 20  $\mu$ L/well for 384-well format) with a minimum of 3 replicates.
2. Add 60  $\mu$ L/well of the LOW Control to empty assay plate wells for 96-well format (or 20  $\mu$ L/well for 384-well format) with a minimum of 3 replicates.
3. Read plate as described in the next section.
4. For each well, calculate the TR-FRET Emission Ratio (e.g., 520 nm/495 nm) by dividing the acceptor emission value (e.g., 520 nm) by the donor emission value (e.g., 495 nm).
5. Average the Emission Ratios for the HIGH Control and separately average the Emission Ratios for the LOW Control.
6. Determine the HIGH to LOW fold-change by dividing the average Emission Ratio for the HIGH by the average Emission Ratio for the LOW.

**Note:** The HIGH/LOW fold-change should be 2–4, depending on the plate reader used. Values below 2 may indicate the instrument is not setup properly and/or lacks sufficient sensitivity for Tb-based TR-FRET.

### ***Reading the Assay Plate***

1. Let assay plate warm to room temperature before reading, if necessary.
2. Set fluorescence plate reader to top/time-resolved read mode (allow plate reader lamp to warm up for at least 10 minutes before making measurements).
3. Remove lid/seal and read plate using the LanthaScreen® Tb TR-FRET instrument-specific filter selection guidelines provided at [www.lifetechnologies.com/instrumentsetup](http://www.lifetechnologies.com/instrumentsetup).

**Note:** Filter bandwidths are critical and cannot be approximated. For instruments using a flash lamp light source, we strongly recommend using 200 flashes for the acceptor channel (e.g., 520 nm) and 100 flashes for the terbium channel (e.g., 495 nm). We also recommend performing plate height (or focal height) optimization for best results.

## Data Analysis

1. For each well, calculate the TR-FRET Emission Ratio (e.g., 520 nm/495 nm) by dividing the acceptor emission value (e.g., 520 nm) obtained for that well by the donor emission value (e.g., 495 nm) obtained for that well. Do **not** average the 520 nm or 495 nm readings and then take the ratio.

**Note:** A common practice employed with TR-FRET data is to multiply the Emission Ratio values by 10,000. This is done to convert Emission Ratio values from decimals to integers for easier graphing and data visualization.

In general, we do not recommend performing background subtraction with TR-FRET data since care must be taken to not inadvertently distort the results. However, in certain cases it may be desirable to perform background subtraction by subtracting the Emission Ratio value obtained for the cell-free control from the Emission Ratios of untreated and treated cell wells.

2. *Optional:* Convert TR-FRET Emission Ratio data into normalized Assay Window values, by dividing each Emission Ratio value determined for an untreated (or DMSO control) cells by the value calculated from the treated cells.

## Additional Assay Performance Data and Troubleshooting Guide

Due to the variety of cell types in which this product may be used, further assay optimization including titration of BacMam Reagent, cell plating density, DMSO tolerance, test compound concentrations and treatment times may be required and is recommended for getting best results in your cell-background of interest. For additional assay performance data and troubleshooting guide, visit [www.lifetechnologies.com](http://www.lifetechnologies.com) and search for A14687 or A14688 to download the **Application Note** for this assay or call the technical support hotline. Protocol and Application Notes are located under the “How to Use” tab on the product page.

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