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Product Information Sheet

BacMam LRRK2-GFP Reagents

Catalog Numbers: A14170, A14191, A14174, A14192

Literature part number: A14170PIS (MAN0005041)

Literature Lot Number: V1

Revision date: 23 August 2011

Material	Amount	Storage	Handling
BacMam LRRK2-GFP Reagent (WT or G2019S)	15 mL or 150 mL	4°C	• Do not freeze • Minimize exposure to ambient light • Use sterile technique • Aliquot to minimize handling, if necessary

BacMam LRRK2-GFP Transduction Guidelines

The first critical experiment is a titration of the BacMam virus in your cell line of interest. 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.

For SHSY5Y cells and other difficult-to-transduce cells, such as human astrocytes and primary neurons, we recommend the following transduction protocol.

Day 1. Plate SHSY5Y cells onto a 6-well plate at 1.5 to 2 million cells/well in growth medium.

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

Day 2. Remove growth medium and wash cells once with PBS with Ca^{2+}/Mg^{2+} (Cat. no. 14040117).

Add 1.5 mL of PBS with Ca^{2+} and Mg^{2+} 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 to 4 hours.

Remove the virus/PBS solution and add 2 mL/well of growth medium containing 0.3X BacMam Enhancer Solution.

Incubate the plate in a 37°C incubator with a humidified atmosphere of 5% CO_2 for 20 to 24 hours.

Day 3. At this point, cells can be harvested and replated onto an assay plate in growth medium without the BacMam Enhancer Solution for another 24 hours. Alternatively, the growth medium in the 6-well plate can be replaced with fresh growth medium without the BacMam Enhancer Solution and the plate incubated for another 24 hours.

Day 4. Perform your assay if you plated the cells on assay plate on Day 3. Alternatively, image the cells on the 6-well plate or harvest the cells for western blot analysis.

***Note*:** For better imaging, we recommend changing the growth medium to PBS prior to imaging.

For easy-to-transduce cells, such as U-2 OS, HEK293, HeLa, and human mammary epithelial cells, we recommend the following the standard transduction protocol. Also see the protocol described for BacMam GFP control (Cat. no. B10383) available at http://probes.invitrogen.com/media/pis/mp10383.pdf.

Note: The expression levels of GFP Control and LRRK2-GFP do not correlate; therefore, the 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.

Day 1. Harvest and resuspend the cells in growth medium.

Add BacMam LRRK2-GFP to the cell suspension at the optimal concentration. For U-2 OS, we recommend 20% (v/v).

Mix gently by inversion. (For some cell types but not U-2 OS, 0.5X BacMam Enhancer solution could be added at this step to increase the expression level)

Transfer cells/BacMam reagent mixture to appropriate cell-culture plates (such as 6-well plates).

Incubate the plates in a 37°C incubator with a humidified atmosphere of 5% CO_2 for 24 to 48 hours.

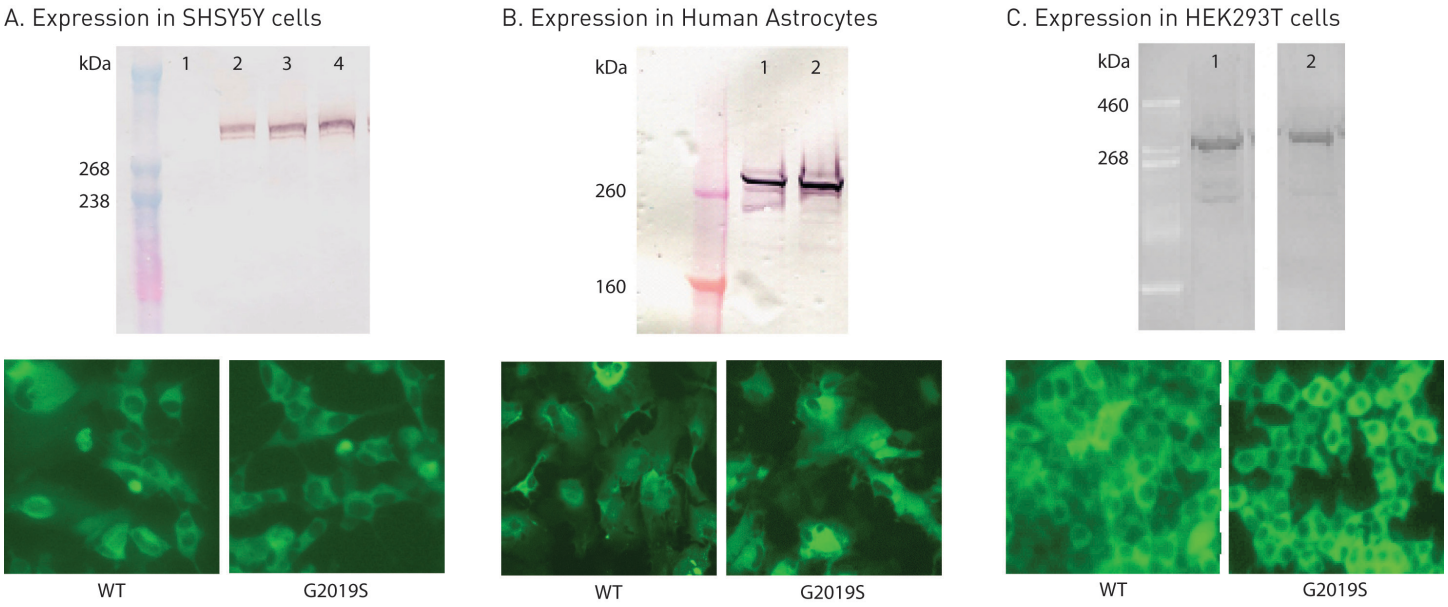
Day 2. At this point, cells can be harvested and replated onto an assay plate in growth medium without the BacMam Enhancer Solution for another 24 hours. Or replace the growth medium in the 6-well plate with fresh growth medium without BacMam Enhancer Solution and incubate the plate for another 24 hours.

Day 3. Perform your assay if you plated the cells on assay plate on Day 2. Or Image the cells on the 6-well plate or harvest the cells for western blot analysis.

Note: The cell plating density and incubation time must be optimized for each cell type.

Representative Data

Figure 1. Western blot analysis and GFP imaging of cells transduced with BacMam LRRK2-GFP. (**A**) SHSY5Y were transduced with 0% (lane 1), 10% G2019S (lane 2), 20% G2019S (lane 3), or 20% WT (lane 4) in a 6-well plate format for ~48 hours. Cells were imaged, harvested, and then lysed with LanthaScreen® Cellular Assay Lysis buffer (Cat. no. A12891). (**B**) Primary Human Astrocytes cells were transduced with 50% WT (lane 1) or G2019S (lane 2) for 2.5 hours, after which the media was replaced and the cells were incubated for 48 hours before imaging and cell lysis. (**C**) HEK293T cells were transduced with 10% WT (lane 1) or G2019S (lane 2) with 0.3X BacMam Enhancer for 48 hours before imaging and cell lysis. Western blot analysis was performed using the anti-LRRK2 antibody (Cell Signaling Technologies, Cat. no. 2567).



Related Products

The following products can be used with cell lysates generated with these BacMam reagents to perform LanthaScreen® Eu Kinase Binding Assays for LRRK2. Lysates containing LRRK2-GFP and LRRK2-GFP G2019S can also be purchased ready-made. For additional information on this technology, visit **www.invitrogen.com/bindingassay**.

Product Name	Catalog Number
LRRK2-GFP Lysate (WT or G2019S)	A14171 or A14172
Kinase Buffer A	PV3189
Kinase Tracer 236	PV5592
LanthaScreen® Eu-anti-GFP antibody	A14173

Technical Support

For additional assistance in using this BacMam Reagent, contact our technical support team at **drugdiscoverytech@lifetech.com** or 760-602-6500 (enter 3 for "know your party's extension", then enter 40266).

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