invitrogen by life technologies

Product Information Sheet

BacMam LRRK2 Reagents

Catalog numbers: A13388, A13389, A13390 **Literature Part Number: A13388.PIS**
Literature Lot Number: V1 Revision date: 8 February 2011

Materials Included

This Product Information Sheet (PIS) covers the following products:

Product	Catalog number	Amount	Storage	Handling
BacMam LRRK2 Reagent	A13388	15 mL	4°C	• Do not freeze • Minimize exposure to ambient light • Use sterile technique • Aliquot to minimize handling, if necessary
BacMam LRRK2 G2019S Reagent	A13389			
BacMam LRRK2 D1994A Reagent	A13390			

BacMam Technology Overview

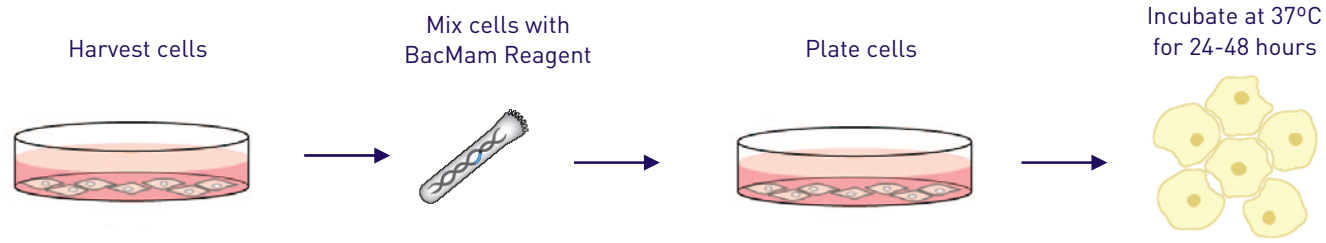
BacMam technology uses a modified baculovirus to efficiently deliver and express genes (in this case, LRRK2) in mammalian cells. The virus is non-replicating in mammalian cells, rendering it safe as a research reagent.

This technology has several advantages over traditional transient methods for heterologous gene expression, including:

- High transduction efficiency across a broad range of cell types, including primary cells, stem cells, and neurons
- Little-to-no observable cytopathic effects
- Reproducible and titratable target gene expression
- Compatibility with simultaneous delivery of multiple genes

Refer to Kost, T.A., et al. *Drug Disc Today* **2007**, *12,* 396-403 for examples of BacMam gene expression in cells. For additional information on BacMam, visit **www.invitrogen.com/bacmam**.

Workflow



Transduction Guidelines

- **Virus titration.** We recommend testing a range of BacMam reagent dilutions (v/v) to determine the optimal percentage of virus to use with your cell line of interest. As a starting point, test 30%, 20%, 10%, 2%, and 1% (v/v) concentrations of reagent. We also recommend testing each dilution in the presence and absence of 1X BacMam Enhancer Solution (Invitrogen PV5835), which may increase the transduction efficiency for some cell lines, such as CHO.
- **Easy-to-transduce cells.** For easy-to-transduce cells, such as U-2 OS, HEK293, HeLa, and human mammary epithelial cells, follow the standard transduction protocol on the next page. Begin with cell cultures grown to near confluency under normal growth conditions (e.g., U-2 OS cells should be grown to 60–90% confluency). Confluency of cells may impact results.
- **Difficult-to-transduce cells.** For difficult-to-transduce cells, such as human astrocytes and neurons, we recommend using the transduction protocol supplied with the BacMam GFP Transduction Control (Invitrogen B10383). You can download the protocol from **http://probes.invitrogen.com/media/pis/mp10383.pdf**.
- **Seeding and harvest densities.** For many cell types, such as U-2 OS, a cell seeding density of ~30,000 cells/cm^2 for 3 days with a harvest density of ~0.6 × 10^5 to 1.2 × 10^5 cells/cm^2 is optimal. In general, cells should be transduced at the maximum density at which the cells are still healthy.

Standard Transduction Protocol

1. Harvest cells in growth medium and resuspend in assay medium, using the appropriate conditions for your particular cell line (for U-2OS cells, resuspend at 7×10^5 cells/mL).
2. Add BacMam LRRK2 Reagent to the cells. A typical final concentration of BacMam reagent is 1–30% (v/v). Mix gently by inversion.

   Note: We recommend testing a range of dilutions of BacMam Reagent (v/v) as described in the **Transduction Guidelines**. For U-2 OS cells, we recommend preparing 5 three-fold serial dilutions of the BacMam Reagent in assay media. Then mix 1 mL cells at 7×10^5 cells/mL with 0.4 mL of each BacMam Reagent dilution. This should yield a final cell concentration of 5×10^5 cells/mL and final reagent concentrations of ~30%, 10%, 3%, 1%, 0.3%, and 0.1% (v/v).
3. Transfer the cells/BacMam reageant mixture to appropriate cell-culture plates (such as 6-well plates or 384-well assay plates).

   Note: You may need to determine the optimal seeding density. For U-2 OS cells, we recommend seeding ~10,000 cells/well (~20 µL of 5×10^5 cells/mL mixed with BacMam reagent) in a 384-well plate
4. Incubate plates for 24–48 hours in a humidified incubator at 37°C/5% CO_2. The incubation time should be optimized for each cell type.

Representative Data

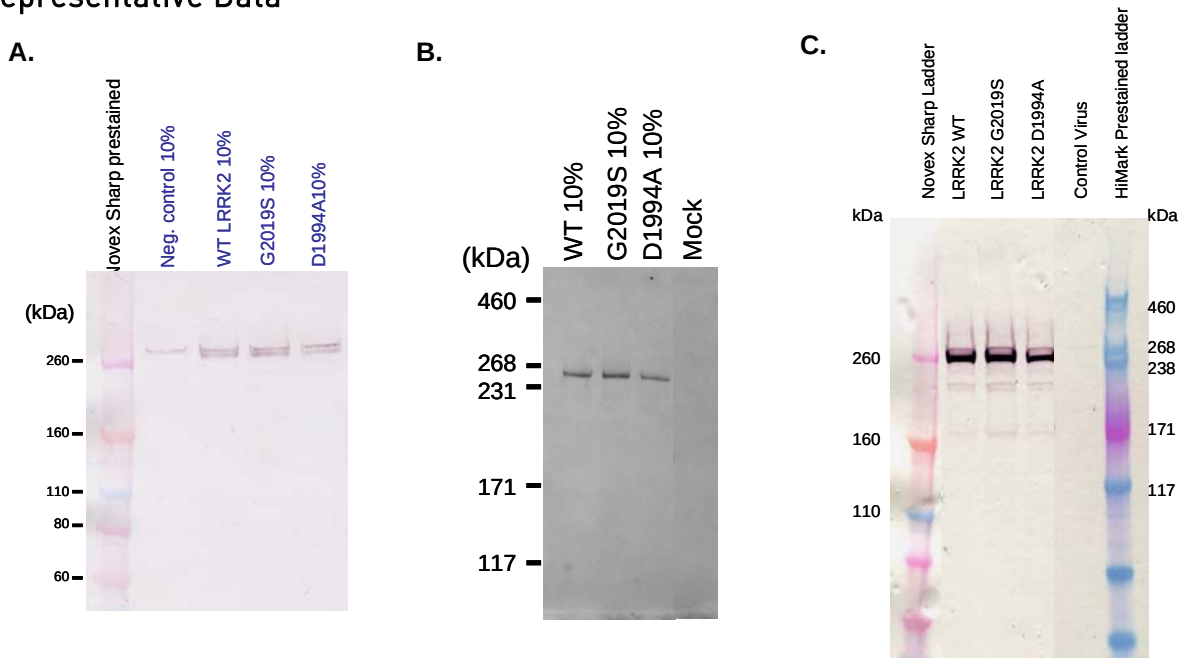


Figure 1. Western blot analysis of BacMam-transduced cells. (**A**) SHSY5Y or (**B**) U-2 OS cells were transduced with 10% virus in 6-well plate format for ~48 hours. Cells were harvested and lysed with LanthaScreen® Cellular Assay Lysis buffer (Invitrogen A12891). (**C**) Primary human astrocytes were tranduced with 50% virus for 2.5 hours, media replaced and incubated for 48 hours before lysis.

Technical Support

For additional assistance in using this BacMam Reagent, please contact our technical support team at **drugdiscoverytech@lifetech.com** or 760-602-6500, extension 40266).

Purchase Notification

Limited Use Label License No: 358: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License No. 21: Bac-to-Bac® and Bac-to-Bac® HT

THIS PRODUCT IS SOLD UNDER PATENT LICENSE FROM MONSANTO FOR RESEARCH PURPOSES ONLY AND NO LICENSE FOR COMMERCIAL USE IS INCLUDED. REQUESTS FOR LICENSES FOR COMMERCIAL MANUFACTURE OR USE SHOULD BE DIRECTED TO DIRECTOR, MONSANTO CORPORATE RESEARCH, 800 N. Lindbergh, St. Louis, Missouri 63167.

Limited Use Label License No. 308: WPRE Element

This product contains the Woodchuck Post-transcriptional Regulatory Element ("WPRE") which is the subject of intellectual property owned by The Salk Institute for Biological Studies, and licensed to Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; and/or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. In addition, any use of WPRE outside of this product or the product's authorized use requires a separate license from the Salk Institute. Life Technologies will not assert a claim against the buyer of infringement of patents owned by Life Technologies and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product or for a Commercial Purpose. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008, Phone (760) 603-7200. Fax (760) 602-6500, or The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, Attn.: Office of Technology Management, Phone: (858) 453-4100 extension 1275, Fax: (858) 546-8093.