BacMam K^+ and Na^+ Channels

Table 1. Contents and storage information.

Material	Amount*	Concentration	Storage	Stability
BacMam reagent (Component A)	20 mL	1X (ready to use)	• 2–6˚C • Protect from light • **Do not freeze**	When stored as directed, the product is stable for at least 6 months.
BacMam enhancer (Component B)	1 vial	Not applicable	2–6˚C	
Dimethyl sulfoxide (DMSO) (Component C)	100 µL	Not applicable	• ≤25˚C • Desiccate	
Number of assays: Sufficient material is supplied for 10 × 96- or 384-well plates based on the protocol below.				
*Cat. no. B10147 contains 10 mL each of Components A and B (BacMam reagent for expressing Kv7.2 and Kv7.3, respectively), 1 vial of Component C (BacMam enhancer), and 100 µL of Component D= (DMSO).				

Introduction

Ion channels are transmembrane proteins that facilitate the flow of ions into and out of cells, thereby controlling the membrane potential and excitability. Sodium ions are concentrated outside of cells along with a net positive charge in the extracellular fluid, and potassium ions are concentrated inside of cells along with a net negative charge in the cytosol. Ion-selective channels open in response to stimuli and conduct electrical currents in and out of cells in accordance with these gradients to regulate many important processes in biology.

There exist an estimated 400 ion channel genes.[1] Functional diversity is further increased by the formation of heteromeric channels from multi-subunit complexes and the interaction of specific channels with accessory proteins, generating a very large number of functional ion channel activities characterized in native tissues.[2] Isolation and recapitulation of these ionic currents in voltage clamp and high throughput screening (HTS) experiments is one major goal of heterologous expression. Functional interrogation remains a major challenge where ion channel subunit stoichiometry plays a critical role in holistic cellular function of the complex.[3]

Ion channels are critically important to a number of cellular functions and processes. They mediate information transfer and control cellular homeostasis. Ion channels underlie nerve impulses and signal transmission across synapses and make up the quantal units of communication in the central nervous system (CNS). In addition, ion channels are involved in wide range of biological processes that require rapid cellular changes, such as muscle contraction, epithelial transport of nutrients and ions, T-cell activation and hormonal release. As a consequence of their diverse biological roles, ion channel dysfunctions (channelopathies) have been associated with many disease states,[1,4] including hypertension, cardiac arrhythmias, diabetes, gastrointestinal disorders, cystic fibrosis, epilepsy, migraine, autism, and pathological pain. Hence, ion channels represent important drug targets in drug discovery and are used for essential cardiac and central nervous system safety tests for developmental drug candidates.

Despite the large number of potential targets and their physiological importance, ion channels remain relatively unexploited as pharmaceutical targets, particularly in comparison to other major therapeutic target classes, such as G-protein coupled receptors and kinases. This has been due to challenges in the development of assays and cellular models suitable for drug discovery efforts with acceptable throughput, robustness and cost.[3, 5-8]

One challenge has been in generating robust **cellular models** that are a requirement for successful experimentation and screening. The traditional approach of stable cell lines that constitutively express ion channels has been difficult to obtain due to toxicity, clonal drift on passage, and other cellular inconsistencies. Investigators are therefore increasingly turning to inducible or transient expression systems to overcome these hurdles.

One of the most promising transient expression systems to emerge in modern drug discovery is BacMam technology.[9-12] BacMam technology is based on the use of an insect cell virus (baculovirus) to efficiently deliver and express genes in mammalian cells (Figure 1). Transgenes under mammalian promoter elements are expressed, while baculoviral genes and their promoters are not recognized. As mammalian cells do not support replication of baculoviruses, transduction is extremely well tolerated and generally lacking in cytopathic effects. The inability of baculoviruses to replicate renders them safe as research reagents, with a biosafety level 1 (BSL-1) classification. BacMam technology has successfully driven discovery campaigns at diverse targets, including ion channels, GPCRs, kinases, and transporters.[3, 13-17] Some of the advantages of BacMam technology for cell-based assays are:

- Efficient transduction of mammalian cell lines, including primary cells and stem cells
- Assay-ready cells from frozen storage of pre-transduced or native cells
- Portability—enables assays in pharmacologically relevant cell types, including primary and stem cells, modulation of expression by varying the dose
- Faster assay development and go/no go decisions by eliminating the need to create and maintain stable cell lines

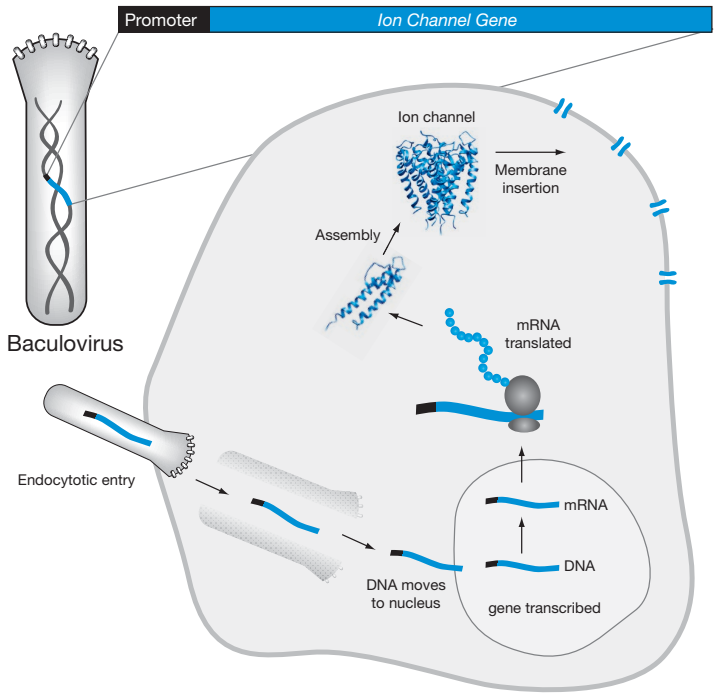

Figure 1. This schematic depicts the mechanism of BacMam-mediated gene delivery into a mammalian cell. BacMam particles are pinocytosed and genes are expressed under a specific mammalian promoter. This process begins within 4–6 hours of transduction and is completed after an overnight period in U-2 OS and other cell types.

Cell types successfully transduced with BacMam K^+ and Na^+ Channel kits include U-2 OS, HEK 293, tSA-201, CHO, HUVEC, and HeLa. We do **not** recommend using cells of hematopoetic origin, because they consistently show very poor BacMam transduction.

BacMam K^+ and Na^+ Channel kits include a virus stock solution (BacMam reagent, Component A) and an enhancer reagent (BacMam enhancer, Component B) for increased expression. The enhancer is reconstituted in DMSO (Component C) prior to use, and added to cells as indicated in the experimental protocol (Figure 2).

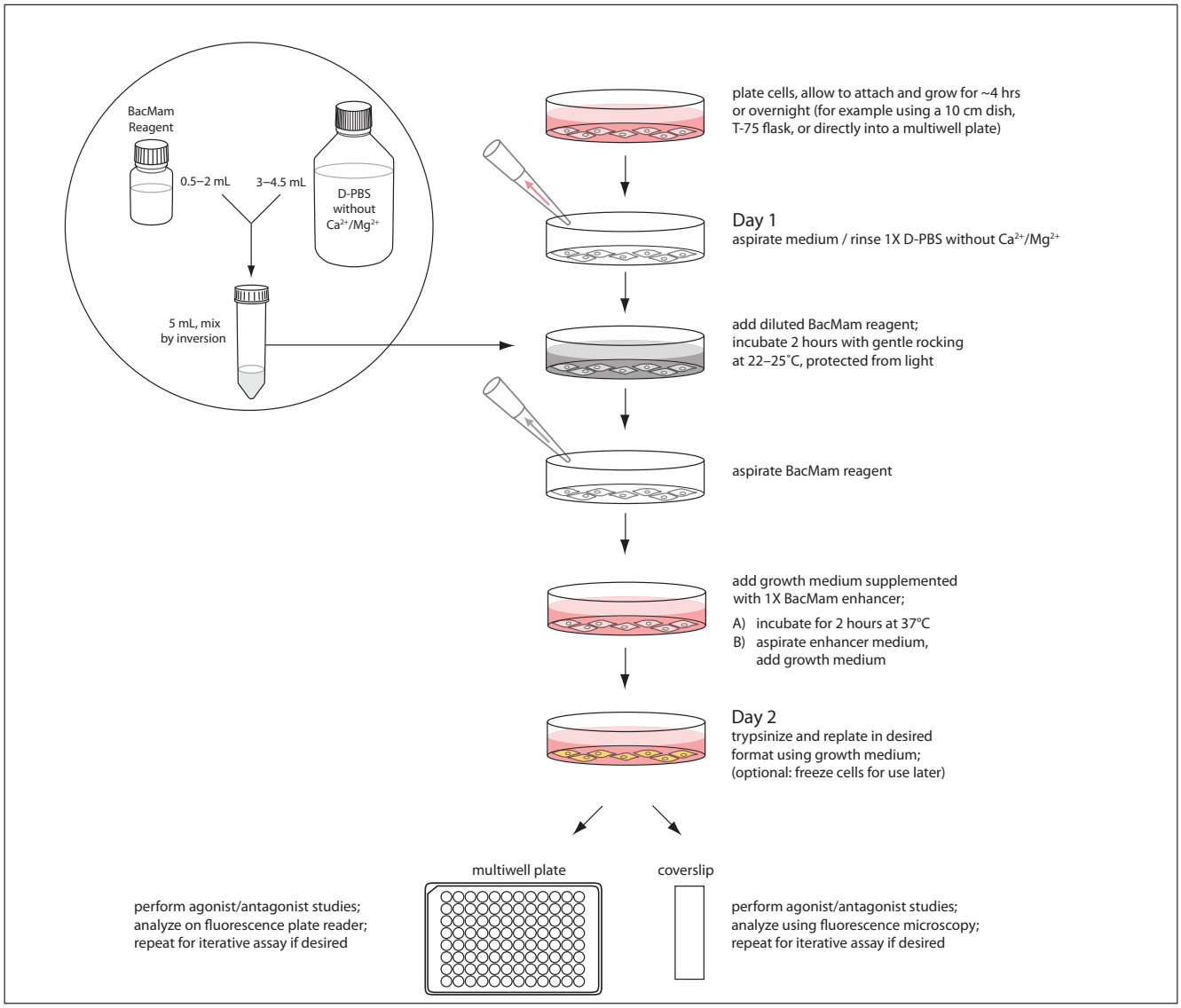

Figure 2. Workflow for BacMam-mediated gene delivery into mammalian cells using the extended protocol.

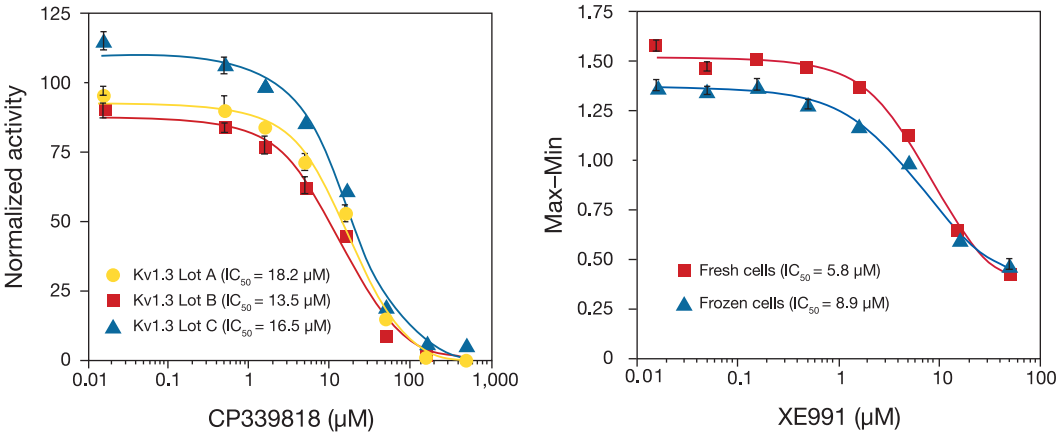

Figure 3. Dose responses with fresh or frozen U-2 OS cells for two K^+ targets in the FluxOR™ assay. Transduced U-2 OS cells expressing Kv1.3 (panel A) or Kv7.2/Kv7.3 (panel B) were split and plated directly for assay or frozen in liquid nitrogen for one week, thawed, and assayed.

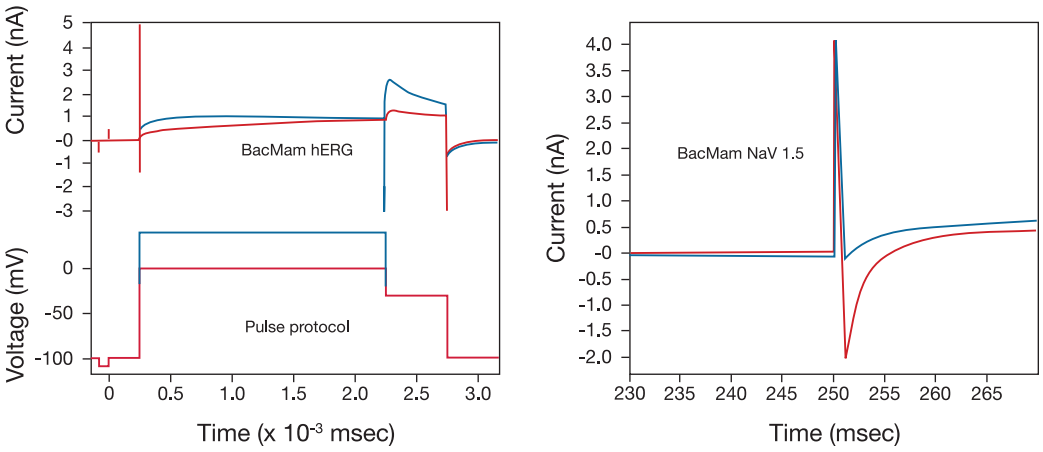

Figure 4. Automated Patch Clamp (APC) measurement of a U-2 OS cell expressing BacMam hERG (Kv11.1) and BacMam Nav1.5 ion channels. U-2 OS cells were co-transduced with 5% vol/vol of each construct as described in the methods. Bottom left panel shows the two step voltage protocol applied to the cells in an Ionworks HT™ instrument. The cells were held at –100 mV and a tail current protocol was applied (blue trace) to measure the outward hERG mediated tail current shown in the left panel. A second step (red trace) was made to 0 mV to capture the peak inward current carried by NaV1.5, shown on an expanded timescale in the right hand panel.

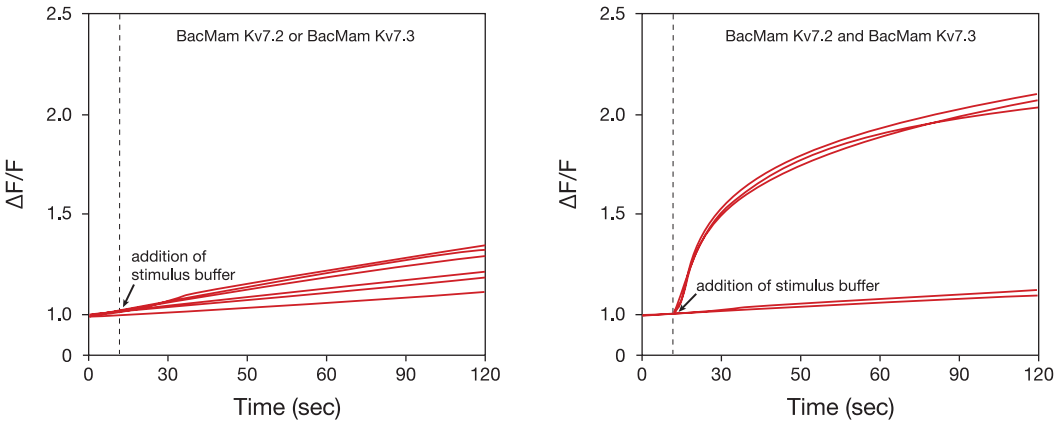

Figure 5. FluxOR™ potassium ion channel assay signatures of Kv7.2 and Kv 7.3, expressed alone (left panel) or together (right panel). Cells were transduced with 5% vol/vol of each construct alone or together as described in the methods, and subjected to the FluxOR™ assay. A control virus (null BacMam) was used to show the background level of activity in the assay (lowest trace). The stimulus was injected 1:5 vol/vol to yield a final added potassium concentration of 10 mM, and a final added thallium concentration of 2 mM.

Before You Begin

Materials Required but Not Supplied

- Mammalian cells and complete growth medium*
- Phosphate-buffered saline (PBS) without Ca^{2+}/Mg^{2+} (Cat. no. 10010)
- TrypLE™ Express dissociation enzyme (Cat. no. 12604-013)
- Hemacytometer and Trypan blue, or the Countess™ Automated Cell Counter
- For high throughput screening (HTS)/microplate assay: Black/clear bottom 96- or 384-well microplates
- *Optional*: 2X freezing medium (80% complete growth medium and 20% DMSO, prepare fresh for each use)
- *Optional*: APC extracellular recording solution for automated patch clamp (APC) assay

* **Note:** Complete growth medium for U-2 OS cells consists of McCoy's 5A medium (Cat. no. 16600-082) and 10% Fetal Bovine Serum (FBS) (Cat. no. 10437-028).

Caution

- BacMam enhancer (Component B) may cause sensitization by skin contact, and is harmful by inhalation and if swallowed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable laboratory protective clothing and gloves while handling this reagent.
- DMSO (Component C) is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of reagents in compliance with all pertaining local regulations.

Experimental Protocols

Transduction with BacMam Ion Channel Reagents—Quick Protocol

This protocol is optimized for use with cryo-preserved U-2 OS cells. See the **Appendix** for how to maintain, propagate, and cryo-preserve U-2 OS cells for optimal BacMam transduction. See Figure 6 for the transduction workflow using the quick protocol.

Day 1

1.1 Thaw, wash, and resuspend cells at ~200,000 viable cells/mL in room temperature complete medium.

1.2 Add BacMam reagent to cells at 5–20% vol/vol (*i.e.*, 0.5–2 mL of BacMam reagent per 10 mL of cells in medium), and mix gently.

Note: If you are transducing with multiple BacMam reagents, pre-mix before adding to cells.

1.3 Incubate for 30–60 minutes at room temperature and **protected from direct light**.

1.4 Dilute cells 2-fold with complete medium to ~100,000 cells/mL, and mix gently.

1.5 Dispense cells into microplates (25 µL per well in 384-well plates, 100 µL per well in 96-well plates), and let the cells settle for 30 minutes at room temperature.

1.6 Transfer microplates to cell culture incubator, and incubate for 12–24 hours at 37°C, 5% CO_2.

Day 2

1.7 Cells are ready for the microplate-based assay.

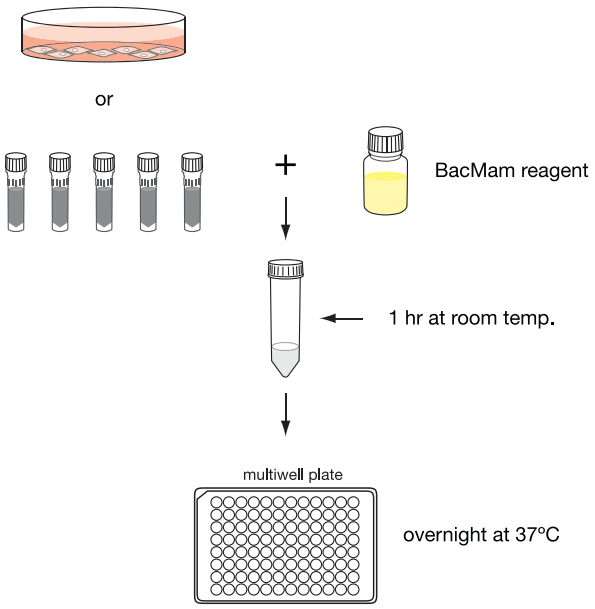

Figure 6. Workflow for BacMam-mediated gene delivery into mammalian cells using the quick protocol.

Transduction with BacMam Ion Channel Reagents—Extended Protocol

This protocol is intended for use with many adherent cell types in a single 75–100 cm^2 culture dish. See Figure 2 for the transduction workflow using the extended protocol.

Day 1

2.1 Mix 0.5–2 mL of BacMam reagent(s) with PBS to a final volume of 5 mL.

Note: If you are transducing with multiple BacMam reagents, pre-mix before adding to cells.

2.2 Remove medium from cells in culture (at 50–70% confluency), and rinse with PBS.

2.3 Remove PBS and replace with 5 mL of BacMam reagent(s) prepared in Step 2.1.

2.4 Incubate with gentle rocking for 2–4 hours at room temperature, **protected from light**.

2.5 Remove the BacMam reagent mixture from cells, and replace with complete medium supplemented with 1X enhancer.

2.6 Incubate for 2–4 hours at 37°C, 5% CO_2.

2.7 Remove the enhancer medium and replace with complete medium. Incubate cells for 12–24 hours at 37°C, 5% CO_2.

Day 2

2.8 Harvest the cells for re-plating into microplates or other assay formats.

2.9 Cells are ready to be assayed 3–24 hours later.

Appendix

This section provides guidlines on how to maintain, propagate, and cryo-preserve U-2 OS cells for optimal BacMam transduction.

General Considerations for U-2 OS Cell Culture

- Grow cells in complete medium at 37°C, 5% CO_2.
- Do not grow cells past 80% confluence or passage 20.
- Seed cells at 20–40% confluence to propagate.
- You do not need to remove the BacMam reagent from the cells prior to your HTS assay.
- U-2 OS cells begin expressing BacMam delivered genes within 6 hours of transduction, and expression levels reach their maximum between 12 to 20 hours post-transduction.
- Plate null BacMam panels as parental controls, and subtract the average signal obtained from these controls as background in your experiment.
- There is no need to remove TrypLE™ Express after 5X or greater dilution in complete medium.

BacMam Gene Delivery for High Throughput Screening with U-2 OS Cells

This protocol describes the expansion and freezing of U-2 OS cells banked for BacMam gene delivery and subsequent high throughput screening (HTS) or automated patch clamp (APC) experimentation. Perform all cell culture, plating, and BacMam reagent handling procedures in a sterile laminar flow tissue culture hood.

Reagents

- Complete growth medium (McCoy's 5A medium plus 10% FBS, store at 4°C)
- PBS without Ca^{2+} or Mg^{2+} (store at room temperature)
- TrypLE™ Express dissociation enzyme (store at room temperature)
- 2X freezing medium (80% complete growth medium and 20% DMSO, prepare fresh for each use)

Thawing U-2 OS cells

3.1 Prepare 10 mL complete growth medium per 2 mL vial of cells.

3.2 Rapidly thaw frozen vials in a 37°C water bath and add to 10 mL of complete medium in a sterile, conical tube.

3.3 Pellet cells by centrifuging at 500 × *g* for 2–5 minutes.

3.4 Pour off the supernatant and evenly resuspend the cells in 5 mL of complete growth medium.

3.5 Determine the number of viable cells per mL using the Countess™ automated cell counter or your method of choice.

3.6 Propagate the cells further or proceed to BacMam gene expression protocols.

Propagating U-2 OS cells

4.1 When resuspended from pellet, immediately transfer the cells into a 15 mL sterile conical tune, and slowly add 10 mL of complete growth medium per 2 mL of cells (*i.e.*, 1:5 vol/vol or greater).

4.2 Gently swirl the cells to mix, and transfer into culture flasks (10 mL of cell suspension per 75 cm^2 culture dish surface area).

4.3 Grow cells in complete medium at 37°C, 5% CO_2, and passage every 3–4 days.

Passaging and harvesting U-2 OS cells

The volumes given in the protocol below are for a 75 cm^2 culture dish. Scale the volumes of PBS, TrypLE™ Express, and complete growth medium according to the surface area of your cell culture vessel.

5.1 Aspirate the spent growth medium from the cells, rinse the cells with 5 mL of PBS without Ca^{2+} or Mg^{2+}.

5.2 To detach the cells, add 1–2 mL of TrypLE™ Express (pre-warmed to 37°C), swirl to cover the cells, and incubate at 37°C for 5 minutes.

5.3 Remove the cells from the incubator, swirl, and incubate at 37°C for an additional 5 minutes or until the cells have completely detached from the culture dish.

5.4 Add 4 mL of complete growth medium, and triturate to suspend the cells evenly.

5.5 Determine the number of viable cells per mL using the Countess™ automated cell counter or your method of choice. Aim to harvest 2–3 million viable cells per 75 cm^2 culture dish.

Freezing U-2 OS cells

6.1 Prepare 1 mL of 2X freezing medium (80% complete growth medium and 20% DMSO) per 2 million cells to be frozen. Aliquot 1 mL of 2X freezing medium into each freezing vial.

6.2 Harvest the cells using TrypLE™ Express as described above (steps 5.1–5.5).

6.3 Pellet cells in a sterile, conical tube by centrifuging at 500 × *g* for 2–5 minutes.

6.4 Pour off the supernatant and evenly resuspend the cells in complete growth medium at 2 million cells per mL.

6.5 Add 1 mL of cell suspension (2 million cells/mL) into each freezing vial containing 1 mL of 2X freezing medium (prepared in step 6.1). Quickly cap the vial and invert several times to mix.

6.6 Store the cells at –80°C overnight in an isopropanol chamber or cell culture freezing device. The next day, transfer the frozen vials to a liquid nitrogen tank for long-term storage. Finished, frozen vials contain 2 million cells each in 2 mL of freezing medium.

BacMam Gene Expression in U-2 OS Cells for HTS and APC

Each frozen vial of 2 million cells prepared as above will provide sufficient material for two microplates or two APC patch plates of cells used at 1 million cells/mL. All BacMam and cell culture reagents should be at room temperature or cooler, including the complete growth medium used in the following protocol.

Reagents

- Complete growth medium (McCoy's 5A medium plus 10% FBS, store at 4°C)
- Frozen vials of U-2 OS cells at 2 million cells per 2 mL vial (prepared in steps 6.1–6.6)
- BacMam reagent (Component A, store at 4°C, protected from light)
- Black/clear bottom 96- or 384-well microplates

Preparing U-2 OS cells from frozen stocks for next day HTS/microplate assay

7.1 Prepare 10 mL complete growth medium per 2 mL vial of cells.

7.2 Rapidly thaw frozen vials in a 37°C water bath and add to 10 mL of complete medium in a sterile, conical tube.

7.3 Pellet cells by centrifuging at 500 × *g* for 2–5 minutes.

7.4 Pour off the supernatant and evenly resuspend the cells in 10 mL of complete growth medium.

BacMam gene delivery and plating cells for HTS

8.1 Remove the BacMam reagent (target or null) from the refrigerator and swirl gently to mix.

8.2 Add 500–2,000 µL (5–20% vol/vol) of BacMam reagent per 10 mL of cells (from step 7.4), tightly cap the tube containing the BacMam reagent/cell mixture, and invert several times to mix.

Note: Pre-mix multiple BacMam reagents in advance before adding to the cells.

8.3 Incubate the BacMam reagent/cell mixture at room temperature for 30–60 minutes, gently rocking, and **protected from light**.

8.4 Add 10 mL of complete growth medium (at room temperature or cooler) and evenly resuspend the cells.

8.5 Using a multi-channel pipettor, dispense 25 µL of cell suspension per well into 384-well microplates, or 100 µL per well into 96-well microplates.

8.6 Allow the cells to adhere for 20–30 minutes at room temperature, and transfer the microplates to a 37°C, 5% CO_2 incubator.

The cells will begin expressing BacMam delivered genes within 6 hours of transduction, and expression levels will reach their maximum between 12 to 20 hours post-transduction.

Using pre-transduced U-2 OS cells

9.1 Thaw or harvest cells from culture as described above.

9.2 Resuspend cells at 200,000 cells/mL.

9.3 Plate 25 µL of cell suspension per well into 384-well microplates, or 100 µL per well into 96-well microplates.

9.4 Allow the cells to adhere for 20–30 minutes at room temperature, and transfer the microplates to a 37°C, 5% CO_2 incubator. The cells will be ready for most HTS assays after 90 minutes incubation at 37°C, 5% CO_2.

BacMam Gene Delivery and Preparing Cells for APC

U-2 OS cells transduced with BacMam ion channel gene products can be used in automated patch clamp (APC) studies. Use the following guidelines as a starting point for APC optimization and screening with BacMam Kv1.3, Kv2.1, hERG, Kir1.1, Kir2.1, or Nav1.5 reagents in U-2 OS cells. This protocol describes the production of ~10 million cells in 10 mL of APC recording solution for use in planar patch experiments.

Reagents

- Complete growth medium (McCoy's 5A medium plus 10% FBS, store at 4°C)
- 4 frozen vials of U-2 OS cells at 2 million cells per 2 mL vial (prepared in steps 6.1–6.6)
- BacMam reagent (Component A, store at 4°C, protected from light)
- APC extracellular recording solution

All BacMam and cell culture reagents, including the complete growth medium used in the following protocol, should be at room temperature or cooler.

Preparing U-2 OS cells for APC

10.1 Rapidly thaw 4 frozen vials in a 37°C water bath.

10.2 Transfer the cells to a sterile 50 mL conical tube, and add 40 mL of complete growth medium.

10.3 Pellet the cells by centrifuging at 500 × *g* for 10 minutes.

10.4 Pour off the supernatant and evenly resuspend the cells in 20 mL of complete growth medium.

BacMam gene delivery

11.1 Remove the BacMam reagent (target or null) from the refrigerator and swirl gently to mix.

11.2 Add 1,000–5,000 µL (5–20% vol/vol) of BacMam reagent per 20 mL of cells (from step 10.4), tightly cap the tube containing the BacMam reagent/cell mixture, and invert several times to mix.

Note: Pre-mix multiple BacMam reagents in advance before adding to the cells.

11.3 Incubate the BacMam reagent/cell mixture gently rocking at room temperature for 30–60 minutes, **protected from light**.

11.4 Evenly split the mixture into two 50 mL conical flasks and add 20 mL of complete growth medium to each flask.

11.5 Pellet the cells by centrifuging at 500 × *g* for 10 minutes..

11.6 Thoroughly and evenly resuspend each cell pellet in 30 mL of complete growth medium and transfer the cell suspensions into two 225 cm^2 cell culture flasks.

11.7 Incubate at 37°C, 5% CO_2 for 12 to 20 hours.

Harvesting U-2 OS cells for APC

12.1 Aspirate the spent growth medium, and rinse the cells with 15 mL of PBS without Ca^{2+} or Mg^{2+}.

12.2 To detach the cells, aspirate the PBS, add 5 mL of TrypLE™ Express (pre-warmed to 37°C), swirl to cover the cells, and incubate at 37°C for 5 minutes.

12.3 Remove the cells from the incubator, swirl, and incubate at 37°C for an additional 5 minutes or until the cells have completely detached from the culture dish.

12.4 Add 20 mL of complete growth medium, and thoroughly triturate to resuspend the cells evenly.

12.5 Transfer the cells to a sterile 50 mL conical tube, and incubate at room temperature for 30 minutes, gently rocking.

12.6 Pellet cells by centrifugation at 500 × *g* for 5–10 minutes.

12.7 Pour off the medium, and thoroughly triturate and resuspend the cells in 10 mL of APC extracellular recording solution.

12.8 Pellet cells by centrifugation at 500 × *g* for 5–10 minutes, and pour off the supernatant.

12.9 Resuspend the cells in 5 mL of APC extracellular recording solution, and determine the number of viable cells per mL using the Countess™ automated cell counter or your method of choice.

12.10 Dilute the cells to 1 million viable cells per mL (or desired concentration) for APC measurements.

References

1. Adv Genet 64, 81 (2008); **2.** Ionic Channels of Excitable membranes, Sinauer Associates, Inc. (1992); **3.** Expression and Analysis of Recombinant Ion Channels, Wiley-VCH, pp. 79–110 (2006); **4.** Hum Mol Genet 11, 2435 (2002); **5.** Receptors Channels 8, 269 (2002); **6.** Assay Drug Dev Technol 2, 543 (2004); **7.** Curr Pharm Design 12, 397 (2006); **8.** Nature Chem Biol 3, 466 (2007); **9.** Nature Biotechnol 23, 567 (2005) ; **10.** Drug Disc Today 12, 396, (2007); **11.** Expert Opin Drug Discov 2, 1669 (2007); **12.** Gene and Cell Therapy: Therapeutic Mechanisms and Strategies, CRC Press, pp. 263–291 (2009); **13.** Receptors Channels 8, 99 (2002); **14.** Receptors Channels 10, 99 (2004); **15.** J Biomol Screen 10, 715 (2005); **16.** Prot Expr Purif 47, 591 (2006); **17.** J Pharmacol Exp Ther 321, 1183 (2007).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
B10146	BacMam Kir2.1 *for 10 microplates*	1 kit
B10147	BacMam Kv7.2 and Kv7.3 *for 10 microplates*	1 kit
B10331	BacMam Kv1.1 *for 10 microplates*	1 kit
B10332	BacMam Kv1.3 *for 10 microplates*	1 kit
B10333	BacMam Kv2.1 *for 10 microplates*	1 kit
B10334	BacMam Kir1.1 *for 10 microplates*	1 kit
B10335	BacMam Nav1.5 *for 10 microplates*	1 kit
B10341	BacMam Nav1.2 *for 10 microplates*	1 kit
Related Products		
B10019	BacMam hERG *for 10 microplates*	1 kit
B10033	BacMam hERG *for 100 microplates*	1 kit
C10130	Cellular Lights™ Null (Control)	1 kit
F10016	FluxOR™ Thallium Detection Kit *for 10 microplates*	1 kit
F10017	FluxOR™ Thallium Detection Kit *for 100 microplates*	1 kit
F10471	Fluo-4 Direct™ Calcium Assay Kit, Starter Pack	1 kit
F10473	Fluo-4 Direct™ Calcium Assay Kit, High-Throughput Pack	1 kit
F14202	Fluo-4, AM *packaged for high-throughput screening*	5 × 1 mg
P10020	PowerLoad™ concentrate, 100X	5 mL
P10229	Premo™ Halide Sensor *for 10 microplates*	5 mL
P36400	Probenecid, water soluble	10 × 77 mg

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
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Fax: (541) 335-0305
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Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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