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Product Information Sheet

BacMam ERK2 [pThr185/pTyr187] Cellular Assay

Catalog Number: A12888
Literature Lot Number: V1

Literature Part Number: A12888.PIS
Revision date: 10 January 2011

FAST FACTS

For first-time BacMam users, we recommend using cells like U-2 OS (ATCC®, HTB-96™), which can be transduced exceptionally well.

BacMam Enhancer Solution (PV5835) is **not** required for this assay using **U-2 OS** cells. However, **it is highly recommended when using CHO cells** (see quick protocol below).

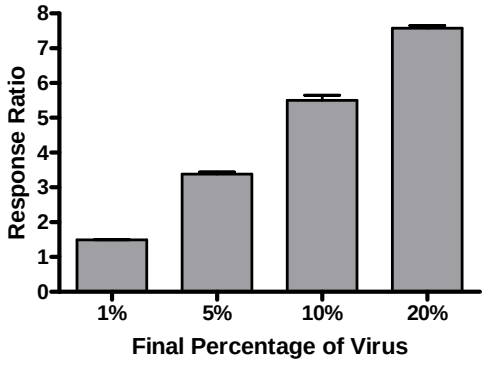
Optimal Virus %: We recommend that you perform a titration of the BacMam ERK2 Reagent to determine the optimal percentage of virus for your transduction in your cell background of interest or when you receive a new lot of virus. Select the lowest percentage of BacMam Reagent that yields the largest assay window (response ratio). See example below.

Kit Components	SKU#	Amount	Storage	Handling
BacMam ERK2 Reagent	A12889	2 × 25 mL	4°C	• Do not freeze • Minimize exposure to ambient light • Use sterile technique • Aliquot to minimize handling,
LanthaScreen® Tb-anti-ERK [pThr185/pTyr187] Antibody	PV5833*	10 µg	–20°C	Aliquot if necessary to avoid multiple freeze/thaw cycles
BacMam Enhancer Solution (1000X)	PV5835	150 µL	–20°C	Aliquot if necessary to avoid multiple freeze/thaw cycles
6X LanthaScreen® Cellular Assay Lysis Buffer	A12891	6 mL	4°C	Supplement with inhibitors and antibody

*To order additional antibody, use catalog no. PV5269 (25 µg amount).

Titration of BacMam ERK2 Reagent in GeneBLAzer® 5HT1A-Gα15-NFAT-bla CHO-K1 cells (Invitrogen catalog no. K1712)

(Detection of ERK2 phosphorylation induced by 5-HT)



Additional Materials Required, but not provided	Source	Part #
Positive Control GPCR agonist **We recommend:** 5-HT (if using CHO cells expressing 5-HT1A)	Sigma	H9523
Positive Control Growth Factor **We recommend:** EGF (if using U-2 OS)	Invitrogen	PHG0314
Cell Line of Interest	Various	Various
DMSO	Fluka	41647
Protease Inhibitor	Sigma	P8340
Phosphatase Inhibitor	Sigma	P0044
White tissue culture-treated, 384-well assay plates	Corning	3570
Fluorescence plate reader with top-read and TR-FRET capability	www.invitrogen.com/instrumentsetup for details	
Optional: Clear-bottom, tissue- culture treated, 384-well plates	Corning	3712

Detailed Protocols and Additional Assay Performance Data Available

Please visit www.invitrogen.com and search for A12888 to download the full protocol and validation packet for this assay.
Protocols and validation packets are located under the "How to Use" tab on the product page.
Validation Packets include assay performance under variable experimental conditions.

Technical Support

For additional assistance in running this BacMam-enabled Cellular Assay, please contact our technical support team at drugdiscoverytech@invitrogen.com or 760-603-7200 (enter 3 for "know your party's extension", then enter 40266).

Quick Assay Protocol for GPCR-mediated ERK2 phosphorylation in CHO cells

The following protocol is developed for a CHO cell line over-expressing the serotonin receptor HTR1A. Conditions such as plating density, stimulant, or stimulation time may need to be optimized for CHO cell lines expressing different GPCRs. Phosphorylation of ERK at [Thr185/Tyr187] can be induced in CHO cells with either 10% serum or 100 nM PMA.

<table>
<tr><th></th><th></th><th>Cell-Free Control Wells</th><th>Unstimulated Control Wells</th><th>Stimulated Control Wells</th><th>Test Compound Wells</th></tr>
<tr><td rowspan="2">BacMam Transduction</td><td>Step 1
Grow and Transduce Cells</td><td colspan="4">• Grow cells in Growth Medium* to 90–100% confluence (~1–2 × 10^5 cells/cm^2).
• Perform five 3-fold serial dilutions of BacMam reagent in PBS with Ca^{2+} and Mg^{2+}.
• Remove media from cells and add the serial diluted BacMam virus.
• Incubate at room temperature protected from light for 3–4 hrs.</td></tr>
<tr><td>Step 2
Add Enhancer and Incubate Cells</td><td colspan="4">Remove virus and add Growth Medium* plus 1X BacMam Enhancer Solution. Incubate the plate at 37°C/5% CO_2 for 20–24 hours.</td></tr>
<tr><td rowspan="9">LanthaScreen® Assay</td><td>Step 3
Harvest Cells</td><td colspan="4">Harvest cells, wash once with Assay Medium** and resuspend in Assay Medium at 0.75 × 10^6 cells/mL (you need to determine the optimal cell density for your cell line).</td></tr>
<tr><td>Step 4
Plate Cells</td><td>20 µL/well Assay Media only</td><td colspan="3">20 µL transduced cells/well (about 15,000 cells/well), quick spin of the plate</td></tr>
<tr><td>Step 5
Serum-starve and Incubate Cells</td><td colspan="4">Incubate the plate at 37°C/5% CO_2 for 4 hours</td></tr>
<tr><td>Step 6
Prepare Complete 6X Lysis Buffer</td><td colspan="4">To 1 mL 6X Lysis Buffer, add 30 µL of 100x protease inhibitor, 30 µL 100x phosphatase inhibitor , and Tb-anti-ERK2 [pThr185/pTyr187] Antibody to 12 nM .</td></tr>
<tr><td>Step 7
Add Agonist</td><td colspan="2">10 µL/well of 0.3% DMSO in Assay Media</td><td>10 µL/well of 3X 5-HT (900 nM) in Assay Media</td><td>10 µL/well of 3X Test Compound in Assay Media</td></tr>
<tr><td>Step 8
Stimulate Cells</td><td colspan="4">Incubate the plate at 37°C/5% CO_2 for 6–8 minutes</td></tr>
<tr><td>Step8
Add Lysis Buffer (including Tb-Ab)</td><td colspan="4">Add 6 µL/ well of Complete 6X Lysis Buffer to each well</td></tr>
<tr><td>Step 9
Cell Lysis/Assay Equilibration</td><td colspan="4">Incubate plate for ~3 hours at room temperature in the dark</td></tr>
<tr><td>Step 10
Read Plate and Analyze Data</td><td colspan="4">See LanthaScreen® Detection in the online protocol—Excitation filter: 337 nm (30 nm bandwidth); Emission filters: 490 nm (10 nm bandwidth) and 520 nm (25 nm bandwidth)</td></tr>
</table>

* **Growth Media:** D-MEM Media (Invitrogen 10569) with 10% dFBS, 10 mM HEPES, 0.1 mM NEAA, and 100 U/mL Penicillin/ 100 µg/mL Streptomycin

** **Assay Media:** Opti-MEM® I (Invitrogen 11058) with 0.1% cdFBS (or dialyzed FBS), 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/100 µg/mL Streptomycin

Quick Assay Protocol for Growth Factor-mediated ERK2 phosphorylation in U-2 OS cells

The cell harvesting and plating densities, growth medium, and assay medium must be optimized for your particular cell line(s). The following protocol is developed for U-2 OS cells. It may be applied to many other cell types such as HEK293, HeLa and A549. Conditions may need to be optimized for different cell types.

		Cell-Free Control Wells	Unstimulated Control Wells	Stimulated Control Wells	Test Compound Wells
BacMam Transduction	**Step 1** Grow, Harvest Cells and Transduce	• Grow cells in Growth Medium* to 90-100% confluence (~0.8–1.2 × 10^5 cells/cm^2). • Harvest and wash cells and resuspend in Assay Medium** at 8.4 × 10^5 cells/mL. • Perform five 3-fold serial dilutions of BacMam reagent in Assay Medium. • Add 0.4 mL undiluted or serial diluted BacMam reagent to 1 mL cells to generate a virus titration range of 0.1% to 30% (v/v) final concentration.			
	Step 2 Plate Cells/virus mixture	Add 20 µL/well Assay Media only	Add 20 µL cells and BacMam mixture per well (~12,000 cells/well), and quick spin the plate		
	Step 3 Incubate Cells	Incubate the plate at 37°C/5% CO_2 for 20–24 hours			
LanthaScreen® Assay	**Step 4** Prepare Complete 6X Lysis Buffer	To 1 mL 6X Lysis Buffer, add 30 µL of 100x protease inhibitor, 30 µL 100x phosphatase inhibitor , and Tb-anti-ERK2 [pThr185/pTyr187] Antibody to 12 nM			
	Step 5 Add media or ligand	Add 10 µL/well of Assay Media with 0.3% DMSO		Add 10 µL/well of 3X Agonist in Assay Media with 0.3% DMSO (for EGF, use ~600 ng/mL)	Add 10 µL/well of 3X Test Compound in Assay Media (0.3% DMSO)
	Step 6 Stimulate Cells	Incubate the plate at 37°C/5% CO_2 for 6–8 minutes			
	Step 7 Add Lysis Buffer (including Tb-Ab)	Add 6 µL/well of Complete 6X Lysis Buffer to each well			
	Step 8 Cell Lysis/Assay Equilibration	Incubate plate for ~3 hours at room temperature in the dark			
	Step 9 Read Plate and Analyze Data	See **LanthaScreen® Detection** in the online protocol—Excitation filter: 337 nm (30 nm bandwidth); Emission filters: 490 nm (10 nm bandwidth) and 520 nm (25 nm bandwidth)			

* **Growth Media for U-2 OS Cells:** McCoy's 5A Media with 10% dFBS, 10 mM HEPES, 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/ 100 µg/mL Streptomycin

** **Assay Media:** Opti-MEM® I (Invitrogen 11058) with 0.1% cdFBS (or dialyzed FBS), 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/ 100 µg/mL Streptomycin

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