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ADORA2A/β-arrestin signaling pathway LinkLight™ assay cells

Version (2016)

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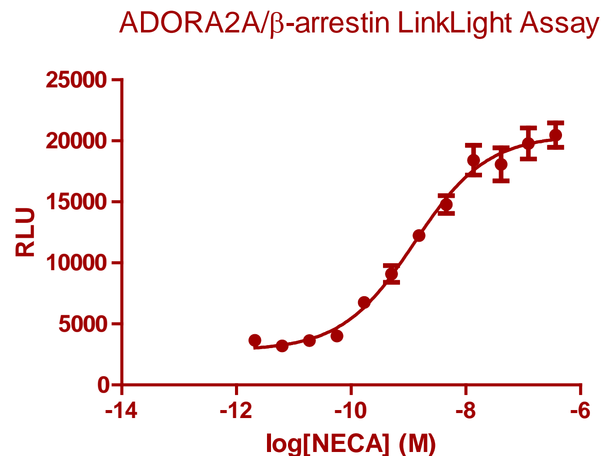
1 Product Description

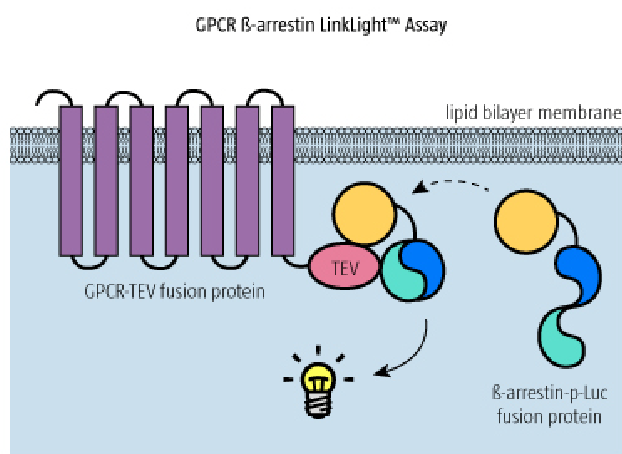
Product Catalog Code: 1051-S
Product Description: Human ADORA2A/β-arrestin LinkLight™ U2OS cells
Human adenosine A2a receptor gene: GeneBank ID_NM_001278497.1 (Consensus CDS: CCD 13826.1)
Human β-arrestin-2 gene: GeneBank ID_NM_004313.3 (Consensus CDS: CCDS11050.1)
TEV protease (Tobacco tech protease): Gene ID_1502321 (modified sequence, proprietary information)
Permuted Firefly Luciferase: (proprietary modified firefly luciferase sequence)
Host cells: U2OS (ATCC number HTB-96) Osteosarcoma; Bone sarcoma from the tibia of a human female.

Representative Data:



2 Technology Principle

GPCR LinkLight™ technology measures GPCR and its signal adaptor protein interactions. An inactive permuted luciferase (pLuc) containing a Tobacco Etch Virus (TEV) protease cleavage site is fused to β-arrestin-2 and a TEV protease is fused to a GPCR. Upon interaction between GPCR and β-arrestin, inactive permuted luciferase is cleaved, the cleaved fragments spontaneously refold, and active luciferase is reconstituted. Highly sensitive luminescent signals produced with the active luciferase are easily detected. The LinkLight assay is not simply another enzyme or protein fragment complementation (EFC or PFC) method. LinkLight technology overcomes the significant drawback of enzyme or protein fragment spontaneous self-complementation. The spontaneous fragment self-complementation caused by high affinity of two fragments increases false interactions and background signals.



3 Materials Provided & Storage Conditions

Human ADORA2A/β-arrestin LinkLight™ U2OS cells.
One cryovial containing ~2x10e6 cells each in 1 mL's of freezing media is shipped on dry ice. Upon receipt, cells should be transferred to liquid nitrogen for storage beyond 24 hours. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C.

4 Materials & Equipment required (not provided)

- Cell culture plates/flasks, pipettes and pipette tips
- 384-well or 96-well microplates (white-walled with solid white or clear bottom, example: BD Falcon Cat.# 353286)
- Luminescence plate reader (EnVision, TopCount, EnSpire, etc. with ultrasensitive lumi-probe)
- LinkLight™ Detection Reagent: ONE-Glo™ , Bight-Glo, Steady-Glo (Promega Cat. No. E6110, E61120, E61130, E2650, E2550) or britelite, neolight (Perkin Elmer Cat. No. 6066761, 6016711)
- Test compounds
- Growth medium McCoy's 5A (Invitrogen, Cat. #16600) and supplements for cells culture
    - McCoy's 5A (Invitrogen, Cat. #16600)
    - 10% FBS (Invitrogen/BRL, Cat. #10082-147)

- 1X Pen/Strep (Invitrogen/BRL, Cat. #10378-016)
- G418 400 µg/ml
- Hygromycin B 100 µg/ml

5 Cell Culture Procedures

5.1. Rapidly thaw the vial of cells by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do not submerge vial in water.
5.2. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5.3. Transfer the vial contents drop-wise into 10 mL of culture medium in a sterile 15-mL conical tube.
5.4. Centrifuge cells at 200 × *g* for 5 minutes.
5.5. Aspirate supernatant and resuspend the cell pellet in 1 mL fresh McCoy's 5A culture media.
5.6. Transfer cells to a flask and culture in a 37ºC incubator at 5% CO2.
5.8 Change the culture media every 3~4 days until 90% confluent. Split the cells by washing one time with PBS (no Ca^{2+}, Mg^{2+}) and adding 0.05% Typsin-EDTA (GIBCO # 25300) and allow the cells to incubate in room temperature until cells detach, add new culture media and transfer viable cells (1: 6 split) to a new culture vessel and place in a 37ºC incubator at 5% CO2.
5.9 Cells can be frozen with 10% DMSO and 20% FBS media.

6 Assay Procedure

The following outlines the procedure for performing the LinkLight™ assay in 384-well plate format (BD Falcon 384-well plate Cat.# 353286).
6.1 Count the cells and adjust cells density with culture media according assay procedure. Seed 20,000 cells per well with 30 µL culture media without G418 and Hygromycin B, incubate cells in a humidified 37°C, 5% CO2 incubator for over 16 hours.
6.2 Gently replace with 15 µL McCoy's 5A media without FBS and antibiotics. (Optional, you may not need to change media if serum has no effect on your compounds or assay. In that case, you can reduce the culture volume).
6.2 Add 5 µL ligand/compound to each well, incubate ~120 minutes in a humidified 37°C, 5% CO2 incubator.
6.3 After incubation, take the plate out and equalize to room temperature for 10 to 15 min.
6.3 Add 20 µL detection reagent Bright-Glo™ reagent (or compatible luciferase detection reagent) to each well, read luminescent light on a luminescence plate reader.

7 Guidelines for Use

We recommend to optimize the assay conditions in your lab first by testing different cell density (10,000 to 20,000 cells/well), compound incubation time (90 min, 120 min, 180 min), serum effect, with and without a reference compound. Please refer to the provider's Firefly Luciferase Detection Kit product information on how to prepare the reagents for use. DMSO concentration should be kept below 1%. The LinkLight cells can be frozen with 20%FBS and 10% DMSO according to standard cell freezing procedures.

8 Tips for Optimal Performance

- Cells density and viability influence S/B ratio.
- Serial dilutions of compound stock are recommended to maintain DMSO at < 1%.
- Use serum-free media for the LinkLight™ assay could reduce background signals.

- Ligand/compound incubation time can be adjusted within 60 to 180 minuets.
- Use clear bottom plate to observe cell growth condition, if you need to monitor cell growth condition.
- Detection reagents should be prepared just prior to use and are light sensitive.
- Optimal signal is generated within 2 to 15 minutes after detection reagent is added to the cells. You can read the luminescent signals multiple times.
- Adjust luminescence plate reader to appropriate sensitivity mode if needed.
- High signal can be obtained by aspirating out media and adding 15 µL LinkLight™ detection reagent, be careful not to suck the cells out.

9 Limited Use License Agreement

The cells (collectively Materials) purchased from BioInvenu are expressly restricted in their use. BioInvenu has developed a GPCR/β-arrestin signaling pathway assay (Assay) that employs genetically modified cells and vectors (collectively, the “Cells”). By purchasing and using the Materials, the Purchaser agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:
9.1. The Materials are not transferable and will be used only at the site for which they were purchased. Transfer to another site owned by Purchaser will be permitted only upon written request by Purchaser followed by subsequent written approval by BioInvenu.
9.2. The Reagents contain or are based upon the proprietary and valuable know-how developed by BioInvenu. Purchaser will not analyze or reverse engineer the Materials nor have them analyzed on Purchaser’s behalf.
9.3. In performing the Assay, Purchaser will use only Reagents supplied by BioInvenu or an authorized BioInvenu distributor for the Materials.
9.4. Purchaser will not use the Cells with any other reagents or substrates, other than the Reagents that are provided by or purchased from BioInvenu or an authorized BioInvenu distributor, in connection with the Materials.
9.5. Purchaser will not transfer the Cells to a third Party.
9.6. Purchaser will not use the Cells for customer services.

10 References

Eishingdrelo H, et al. (2011) A cell-based protein-protein interaction method using a permuted luciferase reporter. Current. Chem. Genomics 5:122-128.

Eishingdrelo H, et al. (2014) ERK and β-arrestin interaction: a converging-point of signaling pathways for multiple types of cell-surface receptors. J. Biomolecular Screen (DOI: 10.1177/1087057114557233).

Hua Li, A.Eishingdrelo, S. Kongsamut, and H. Eishingdrelo. (2016) GPCRs mediate 14-3-3 signal transduction. Signal Transduction & Target Therapy DOI: 10.1038/sigtrans.2016.18

If the purchaser has any further questions regarding the rights conferred with purchase of the Materials, please contact:

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