

PathHunter[®] eXpress GPR39 CHO-K1 β -Arrestin Orphan GPCR Assay

Catalog Number: 93-0338E2

Lot Number:

Contents: 1.25 x 10⁶ cells per vial in 0.1 mL

Background

PathHunter eXpress β -Arrestin Orphan GPCR cells are engineered to co-express the ProLink[™] (PK) tagged GPCR and the Enzyme Acceptor (EA) tagged β -Arrestin. Activation of the GPCR-PK induces β -Arrestin-EA recruitment, forcing complementation of the two β -galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal. These cells have been modified to prevent long term propagation and expansion using a proprietary compound that has no apparent effect on assay performance.

Product Information

Target GPCR: GPR39

Description: G-protein coupled receptor 39

Receptor Family: Class A Orphan

β -Arrestin Isoform: β -Arrestin-2

Accession Number: NM_001508

ProLink[™] Tag: PK1

GPCR Species: Human

Cell Type: CHO-K1

Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

Cell Plating Reagent: AssayComplete[™] Cell Plating 1 Reagent

Functional Performance

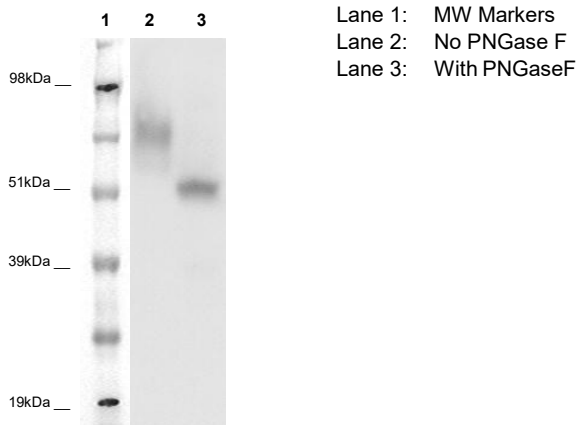


Figure 1. Cell lysates prepared from PathHunter β -Arrestin Orphan GPCR β -Arrestin Cell Lines were treated with PNGase F (Glyko: GKE -5003), run on a SDS-PAGE gel and analyzed. Untreated lane resolves a band of appropriate size corresponding to GPCR-PK fusion protein and the PNGase F treated lane resolves a deglycosylated band indicative of proper expression and folding of GPCR protein.

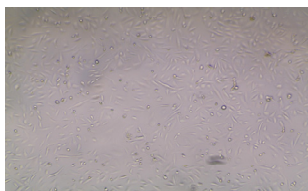


Figure 3. Viability of PathHunter eXpress cells were confirmed by bright field microscopy.

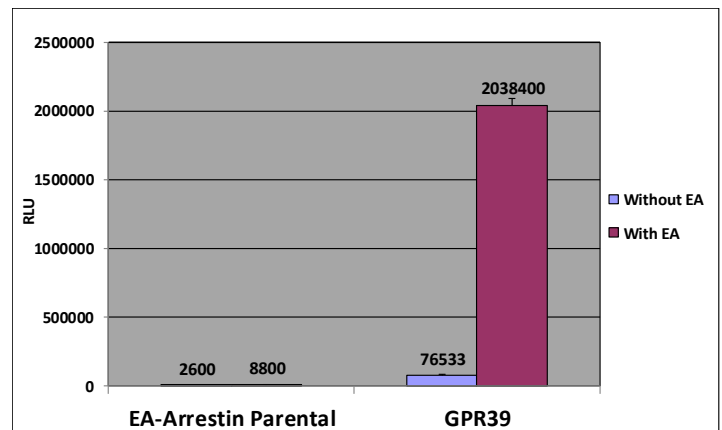


Figure 2. PathHunter eXpress cells were analyzed for basal activity as well as GPCR-ProLink[™] expression by comparing the ratio of signal between untreated cells and cells treated with saturating amounts of exogenous EA, using ProLink[™] Detection Kit (DrX: 92-0006). Signal from complementation of ProLink[™] and EA fragments correlates to the amount of GPCR-PK expression in the cell line.

Important! This assay requires an additional step: Please refer to Additional Protocol Information section.

Additional Protocol Information

ZnCl₂ will strongly inhibit Enzyme Fragment Complementation (EFC) enzyme activity at pharmacologically relevant concentrations, preventing detection of an agonist-induced PathHunter assay signal. Therefore, ZnCl₂ cannot be used as an agonist in this assay to test receptor-mediated arrestin recruitment. However, ZnCl₂ is compatible with typical Calcium flux assays, which do not use EFC for signal detection.

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