PathHunter™ ß-arrestin assay with Mithras LB 940

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Introduction

PathHunter ß-Arrestin Assays offer a generic method that can be applied to any GPCR and is based upon the interaction of arrestin with the GPCR during activation. The PathHunter ß-Arrestin Assay is unique compared to other arrestin assays in that it provides a direct measure of ß-Arrestin binding to the GPCR of interest, unlike imaging assays that detect movement of the arrestin molecule. Thus, the assays are ideal for HTS and may be very useful for deorphanizing novel GPCRs.

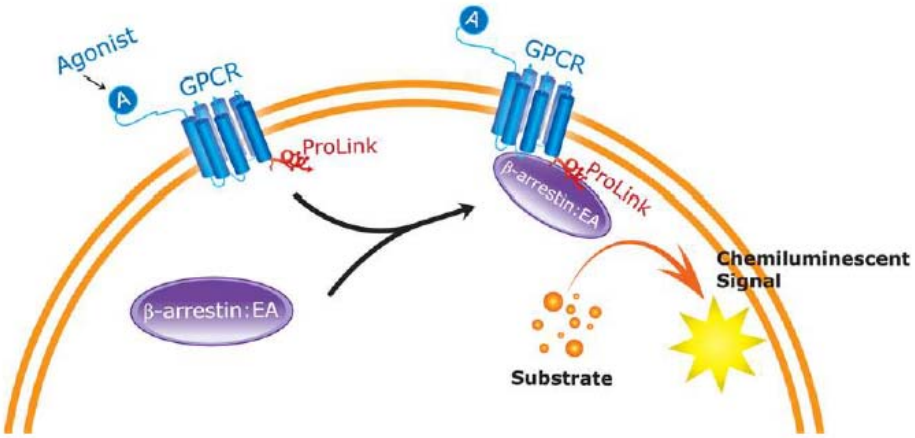

Figure 1: PathHunter™ ß-arrestin principle

The assay detects binding of an agonist to a GPCR of interest by directly measuring ß-arrestin binding to the GPCR. Once bound, complementation occurs between the two ß-galactosidase components: the ProLink tag is fused to the C-terminus of the GPCR of interest; the Enzyme Acceptor (EA) is attached to ß-arrestin. These components interact only when in close

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proximity, forming active ß-gal enzyme that converts substrate to detectable signal.
The EFC approach offers a range of benefits for screening, including signal amplification, high assay windows, robust performance, and a homogenous, single-addition assay format. The chemiluminescent signal generated can easily be read in 96-, 384- or 1536-well microplates with a standard luminometer, so library screening is simple, fast, and cost effective.

The Mithras LB 940 is a multimode plate reader with a unique optical design (DOPS – Dedicated Optical Path System) to ensure optimized performance for the detection technologies implied. These are

- luminescence
- BRET/BRET²
- fluorescence
- UV/VIS absorbance
- fluorescence polarization
- AlphaScreen™
- TRF
- HTRF®

Figure 2: Mithras LB 940 multimode reader

In addition accessory options, e.g. reagent injectors, temperature control and cooled PMT detection units are available. The combination of an unmatched efficiency for luminescence detection including the proprietory crosstalk-reduction design with the reagent injectors make the instrument ideally suited for the PathHunter™ technology.

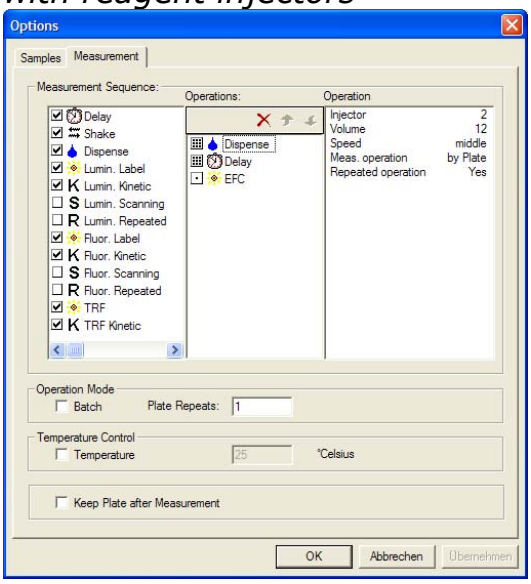
Methods

Assay Protocol:

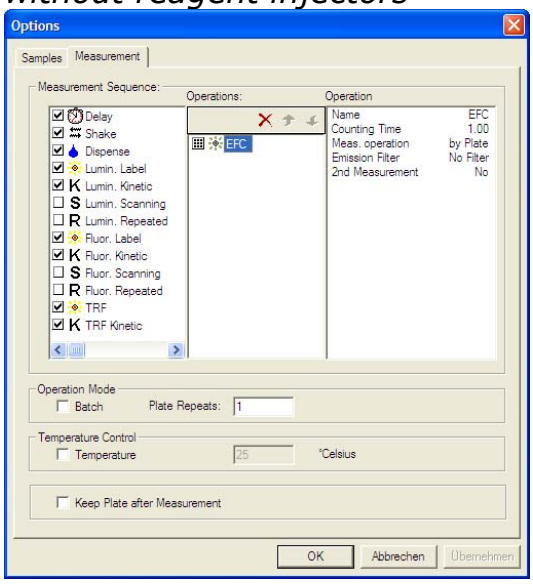
- Seed 20 µL 5000 HEK-CCKAR PathHunter cells into white 384 well solid or clear-bottom microplates
- Incubate overnight
- Add 5 µL serially diluted CCL27 or CCK8-SO4
- Incubate for 90 min at 37 °C
- Add 12.5 µL PathHunter detection reagent
- Incubate for 1 hour at room temperature
- Read in luminescence mode for 1 s per well

Instrument settings:

with reagent injectors



without reagent injectors



Results

The assay read in the Mithras provides S/B ratio of 2.8 and an EC_{50} value of 13 nM.

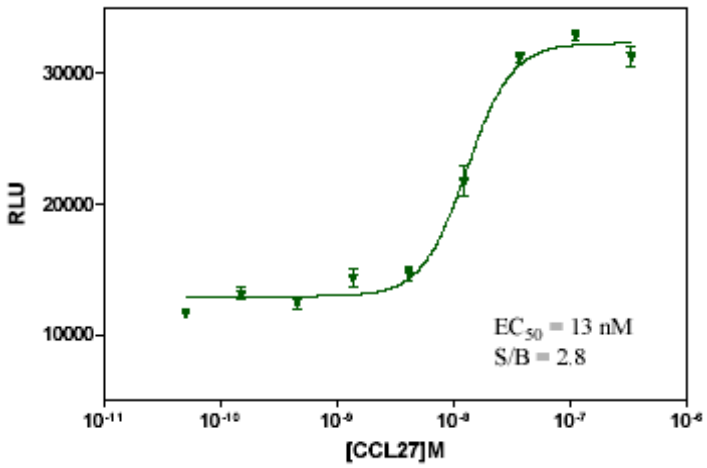


Figure 3: plot of signal vs. CCL27 concentrations

Conclusion

- The Mithras shows excellent S/B values due to its sensitive luminescence detection fully matching the superb sensitivity of the PathHunter™ ß-arrestin assay.
- The assay read on the Mithras provides an EC_{50} of 13 nM.

Materials

- Mithras LB 940, equipped with one reagent injector (Berthold 38099)
- PathHunter™ cAMP HS+ kit (DiscoveRx 93-0001)
- White microplates: 384 well cell-culture-treated or 384 well clear-bottom cell-culture treated or 96 well cell-culture-treated (Berthold 51538) or 96 well clearbottom cell-culture treated (Berthold 24910)
- additonal reagents see kit insert