PathHunter™ ß-arrestin flash assay with Mithras LB 940

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Introduction

With the PathHunter™ Flash Detection Kit, ß-arrestin recruitment by a GPCR can now be detected in 30 seconds. Fast PathHunter ß-arrestin chemiluminescent assays, unlike lengthy reporter gene assays, greatly minimize the opportunity for off-target effects. A ligand-activated, GPCR-arrestin interaction combines two ß-galactosidase fragments, enabling rapid detection in a homogeneous format. The kit is designed for plate readers with onboard fluidics and flash detection mode. Additionally, screening campaigns can be accelerated by conducting ß-arrestin and calcium assays in the same well when using instruments capable of real-time fluorescence and flash chemiluminescence.

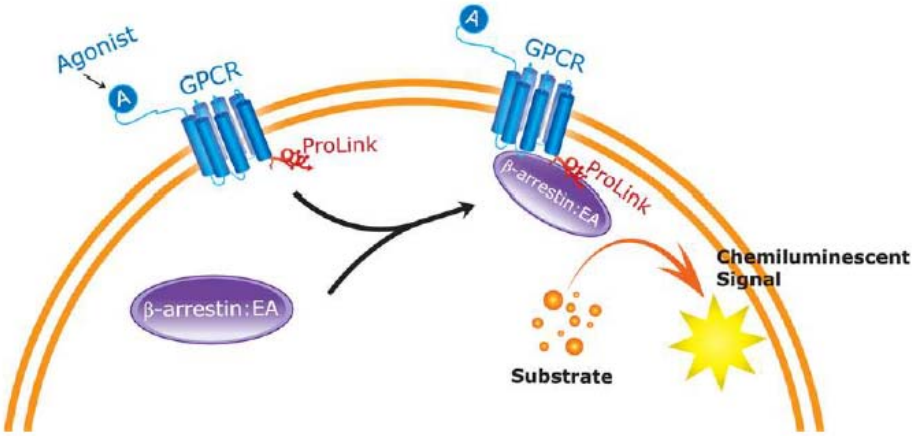

Figure 1: PathHunter™ ß-arrestin principle

The Flash Kit measures ß-gal Enzyme Fragment Complementation (EFC) activity in PathHunter™ Cell Pools and Lines expressing the Enzyme Acceptor

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(EA) and either ProLabel™ or ProLink™ tags-tagged target proteins used in PathHunter ß-arrestin assays. A ligand-activated, GPCR-arrestin interaction combines two ß-galactosidase (ß-gal) fragments, enabling rapid detection in a homogeneous format. The kit is designed for plate readers with onboard fluidics and flash detection mode and has been validated for use in both 96-well and 384-well microplate formats.

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®

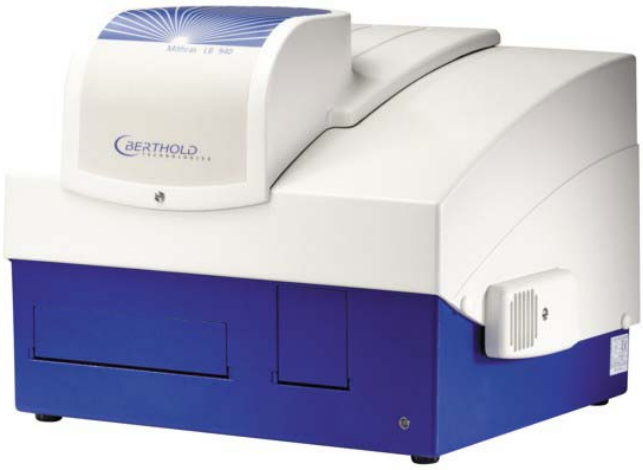


Figure 2: Mithras LB 940 multimode reader

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™ flash assay technology. The possibility to combine the luminescent read-out with a fluorescent read-out allow the combination of flash ß-arrestin and flash Calcium assays in a single well.

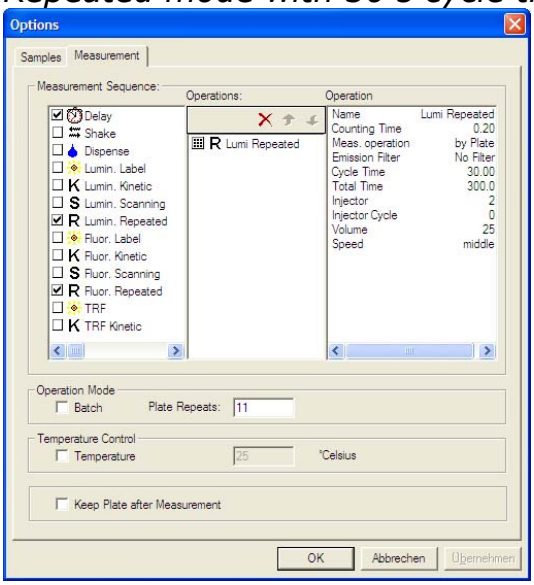
Methods

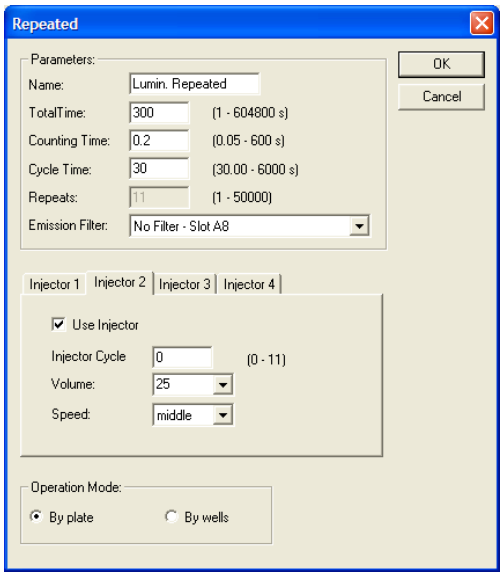
Assay Protocol:

- Seed 20 µL 2000 HEK-CCKAR PathHunter cells into white 384 well solid or clear-bottom microplates
- Incubate overnight
- Add 5 µL serially diluted CCK8-SO4
- Incubate for 60 min at 37 °C
- Add 25 µL PathHunter Flash substrate by using an on-board injector
- Read in luminescence repeated mode for 0.2 s per well with 30 sec intervals (max. 24 wells; with a bigger number of wells the cycle time time for the intervals needs to be extended)
  or
  read in luminescence kinetic mode for 90 sec per well with 1 s counting time
  or
  take a single read-out within 6 min after the substrate has been added (full 384 well plate) or within 3 min (half 384 well plate)

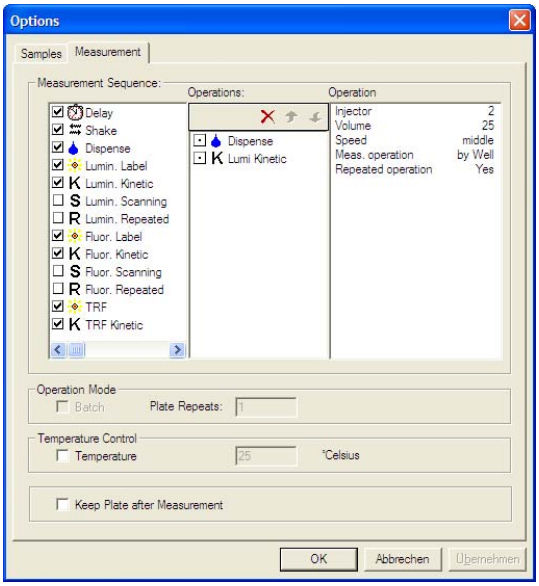
Instrument settings:

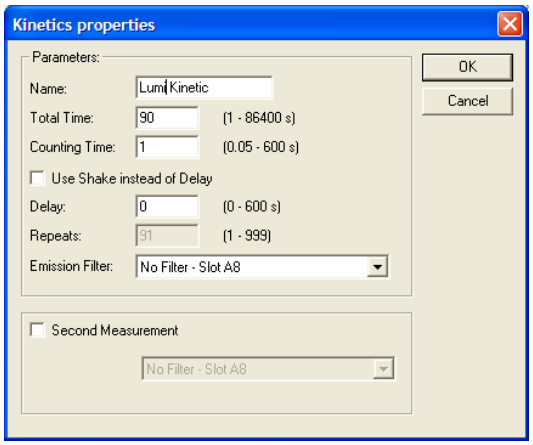
Repeated mode with 30 s cycle time



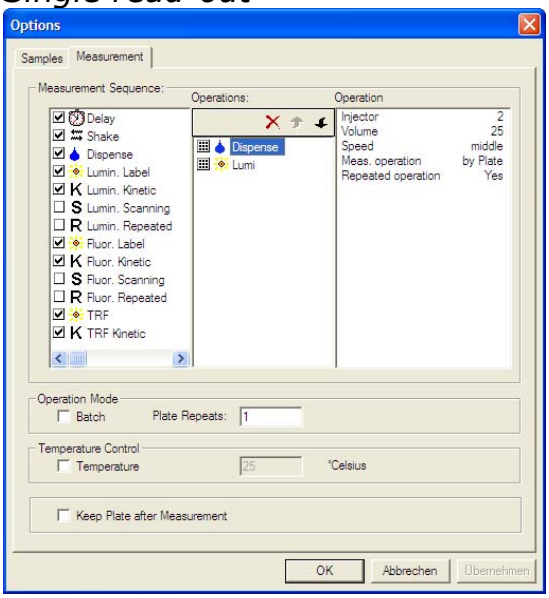


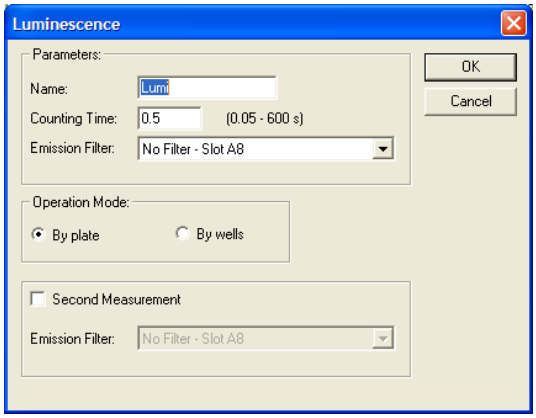
Kinetic mode





Single read-out





For the repeated and kinetic operations the calculation part of the software may be used to determine AUC, peak or slope values.

Results

Within the concentration range chosen there is a clear relation of luminescent signal vs. concentration for all time points after injection monitored. The concentration range selected has been too low for being able to see ligand saturation and derive EC_{50} thereof.

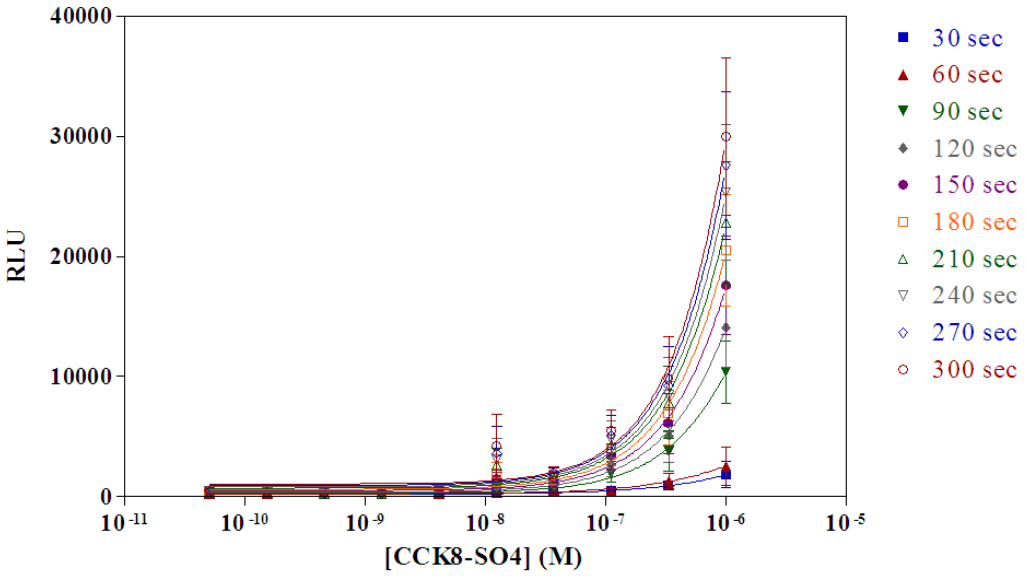


Figure 3: plot of signal vs. CCK8-SO4 concentrations for various times after injection

Conclusion

- The PathHunter™ Flash assay provides the ability to read the signal within much shorther time than with the glow-type format as one incubation step can be omitted.

Materials

- Mithras LB 940, equipped with one reagent injector (Berthold 38099)
- PathHunter™ Flash ß-arrestin kit (DiscoveRx 93-0247)
- 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