



PathHunter™ secretion assay with Mithras LB 940

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Introduction

PathHunter™ products comprise a family of simple, robust cell-based assays and related tools designed to directly measure protein translocation, secretion, degradation, expression, and cell proliferation-without imaging or antibodies. These products may address cell-signaling cascades in which a target protein undergoes nuclear translocation, degradation, or secretion. A variety of targets can be measured, including transcription factors, nuclear hormone receptors, and kinases. Other unique PathHunter assays enable measuring mitotic activity in mammalian cells.

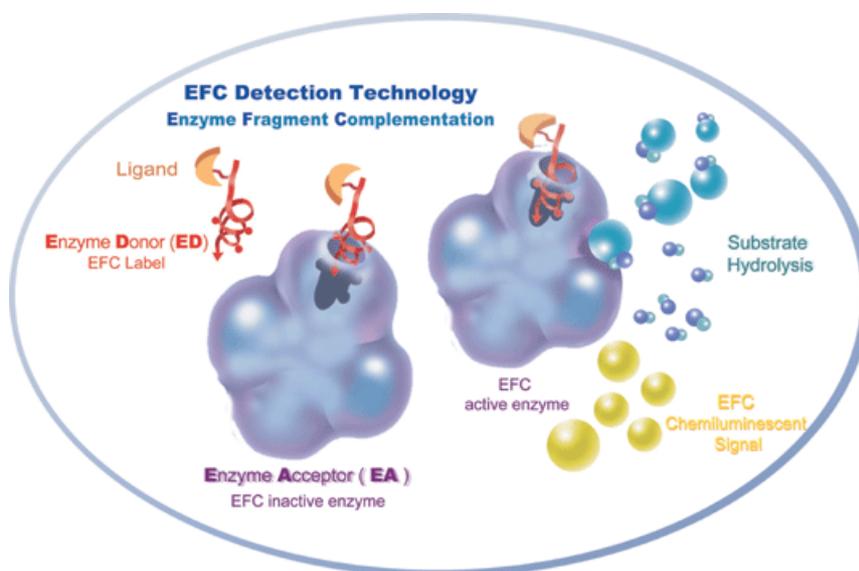


Figure 1: EFC detection principle

PathHunter products feature a novel, non-imaging approach based upon detecting complementation of two β -galactosidase fragments, which

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generates a functional enzyme and a chemiluminescent signal. Consequently, no antibody labeling or imaging is required, and the assays are compatible with standard microplate readers. The basic PathHunter procedure is elegantly simple, and its homogeneous, two-step format offers high-sensitivity chemiluminescent detection.

The PathHunter Secretion assay is a sensitive method to detect secreted recombinant proteins by applying a non-lysis form of detection reagents.

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 proprietary crosstalk-reduction design with the reagent injectors make the instrument ideally suited for the PathHunter™ technology.



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Methods

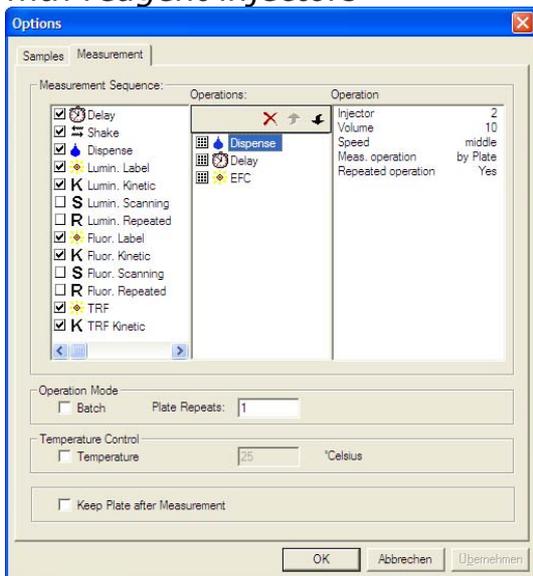
Assay Protocol: 1 hour secretion

- Seed 20 μ L HEK-PL10 cell in amounts ranging from 0 to 5000 into white 384 well solid or clear-bottom microplates
- Replace media with fresh media
- Incubate for 1 h
- Add 5 μ L EA reagent
- Add 5 μ L chemiluminescence substrate
- Incubate for 1 hour at room temperature
- Read in luminescence mode for 1 s per well

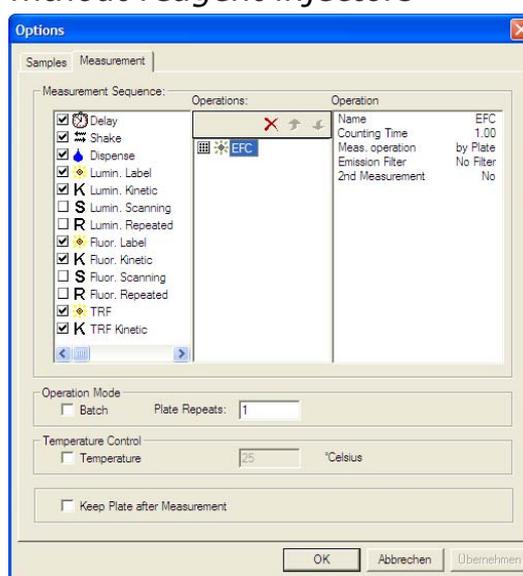
Assay Protocol: overnight secretion

- Seed 20 μ L HEK-PL10 cell in amounts ranging from 0 to 5000 into white 384 well solid or clear-bottom microplates
- Replace media with fresh media
- Incubate overnight
- Add 5 μ L EA reagent
- Add 5 μ L chemiluminescence substrate
- Incubate for 1 hour at room temperature
- Read in luminescence mode for 1 s per well

Instrument settings: with reagent injectors



without reagent injectors



Note: When using the injectors in the 384 well format to dispense EA reagent and CL substrate combine both reagents in a reservoir and inject the mixture of both as the minimum volume of the injectors is 10 μ L.



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Results

We see a linear response (luminescent signal versus cell number) for both secretion times. Overnight secretion results in a bigger luminescent signal for the same cell number.

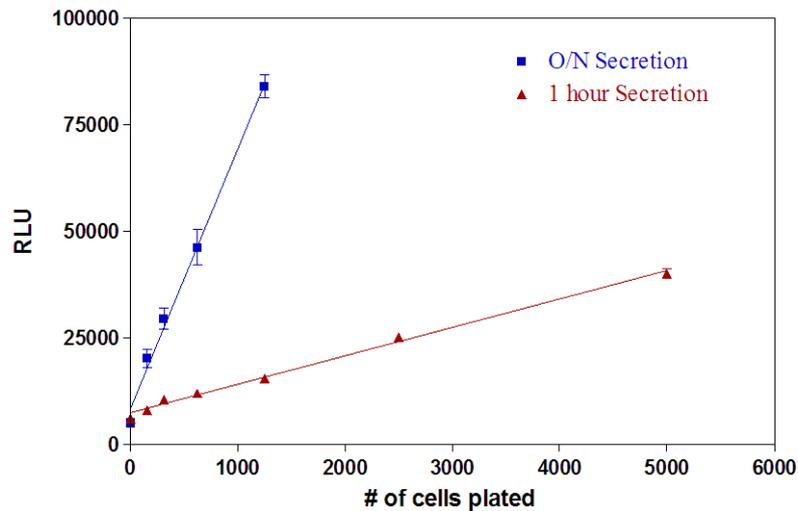


Figure 3: plot of signal vs. cell number for 1 hour and overnight secretion

Conclusion

- PathHunter™ is a highly sensitive method to detect secreted recombinant proteins.
- Mithras is an excellent instrument to enhance the assay's sensitivity.



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Materials

- Mithras LB 940, equipped with one reagent injector (Berthold 38099)
- PathHunter™ EA reagent (DiscoverX)
- PathHunter™ CL substrate (DiscoverX)
- Cell line constitutively expressing PL10 (DiscoverX)
- 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)
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AlphaScreen™ is a trademark of PerkinElmer
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