Bioluminescence Imaging of Live Trout for Virus Detection using EnduRen™ Live Cell Substrate

ABSTRACT We review a novel method for bioluminescence imaging of live fish. In order to study the entry route and pathogenesis of a member of the virus family *Rhabdoviridae* in young trout, Harmache *et al.* used a unique live fish imaging system and bioluminescence. They were able to show locations of viral entry and replication by examining fish at various times postinfection and using a variety of pathogenic and nonpathogenic *Novirhabdoviridae* isolates.

Kari Kenefick, M.S., Promega Corporation

Harmache, A. *et al.* (2006) *J. Virol.* **80**, 3655–9.

Recent advances in bioluminescence imaging allowed the authors to investigate both the sites of viral entry and of viral replication by imaging live fish at various times postinfection.

INTRODUCTION

In the paper "Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for *Novirhabdovirus*" (1) the authors used EnduRen™ Live Cell Substrate to study viral entry and spread in yearling trout.

The virus studied was IHNV, infectious hematopoietic necrosis virus, which belongs to the genus *Novirhabdovirus* of the family *Rhabdoviridae*. This virus causes serious, lethal disease in trout. However, it is not known where the virus enters trout, nor has it been definitively determined where the sites of viral replication are. Recent advances in bioluminescence imaging allowed the authors to investigate the site of viral entry as well as sites of viral replication due to the fact that live animal imaging could be performed at various times post-infection and over the entire fish.

EnduRen™ Live Cell Substrate[a–c] (Cat.# E6481, E6482) generates *Renilla* luciferase luminescence from live cells under normal growth conditions. This substrate produces very stable luminescence with high signal readings and low autoluminescence, allowing luminescence measurement for at least 24 hours after substrate addition. EnduRen™ Substrate can be used in a variety of cell analysis techniques, including reporter gene analysis.

For these studies, the authors created a recombinant IHNV, rIHNV$_{LUC}$, that expressed the *Renilla reniformis* luciferase reporter gene. Expression of *Renilla* luciferase was initially tested by monitoring rIHNV$_{LUC}$-infected EPC cell lysates with a luminometer. In EPC cells, luciferase expression was detected as early as 2 hours postinfection with luminescence intensity increasing to a peak at 36 hours.

To determine whether insertion of the *Renilla* luciferase cassette into the virus DNA affected pathogenicity, 75 juvenile trout were exposed, by bath immersion, to one of three viruses; wild-type rIHNV, rIHNV$_{LUC}$, or viral isolate IHNV 32–87. Daily mortality counts were taken for 20 days post-virus exposure and showed that the three isolates had similar degrees of pathogenicity.

Experimental infection was then repeated, and infected fish were sampled during days 0–12 post-infection. Various organs (spleen, kidney, heart and liver) were collected each day, homogenized and monitored for luciferase activity. Spleen and kidney showed active virus replication as early as day 3, while no significant luciferase activity was detected during days 0–2 postinfection.

To follow viral spread in the fish in a noninvasive manner, the researchers infected trout, then added EnduRen™ Live Cell Substrate to the fish tank water. Infected fish were collected on day 4, anesthetized and subjected to CCD camera imaging to detect luminescence. Active viral replication was seen in the oral cavity, esophagus/cardiac stomach region, the pyloric caeca, kidney and spleen, plus the dorsal fin. One fish was sacrificed and its skin removed before CCD imaging to show that luminescence was being emitted from locations inside the body versus simply being present on the exterior of the fish.

To examine the kinetics of virus replication after waterborne infection, fish were infected by bath immersion. Beginning 8 hours postinfection, fish were collected and transferred to a tank containing the water-soluble EnduRen™ Substrate, then exposed to imaging with a CCD camera. The resulting images show viral replication at early times (8 and 16 hours postinfection; images not shown here) at the fin bases, and later (24 and 40 hours) involving internal organs. However, the fin bases still showed luciferase luminescence at the later time points.

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Although IHNV is frequently an acute, lethal infection, 10–20% of trout typically survive the infection. The authors used the surviving trout to examine viral spread 3 weeks after infection. Four trout plus one mock-infected trout were examined postinfection; the infected trout had a wide range of viral distribution and viral load. Some fish showed systemic viral spread, while others showed bioluminescence localized to the fins.

The authors had previously reported a recombinant IHNV-deltaNV isolate (rIHNV-deltaNV) that was not pathogenic for fish (2). To this virus they added the *Renilla* luciferase expression cassette, then used the virus to infect fish by bath immersion. After 3 weeks the fish appeared healthy with no mortalities reported. Bioluminescence imaging of these $rIHNV_{LUC}$-deltaNV-infected fish showed a limited viral replication localized exclusively to the fin bases, reminiscent of the early (8-hour postinfection) data for the $rIHNV_{LUC}$ fish. This data, as well as data from work with other fish pathogens, supports the authors' hypothesis that fin bases are entry points for pathogens, including IHNV (3).

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REFERENCES

1. Harmache, A. *et al.* (2006) *J. Virol.* **80**, 3655–9.
2. Thoulouze, M.I. *et al.* (2004) *J. Virol.* **78**, 4098–107.
3. Buchmann, K. (1998) *Folia Parasitol.* (Prague) **45**, 312–8.

ORDERING INFORMATION

Product	Size	Cat.#
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485

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[a]Patent Pending.

[b]Certain applications of this product may require licenses from others.

[c]This product does not convey a license to use recombinant *Renilla* luciferase under U.S. Pat. Nos. 5,292,658, 5,418,155 and related patents. Promega sells licensed *Renilla* luciferase vectors, which may be used in conjunction with this product.

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ALSO IN THE LITERATURE

Dinh, D., et al. (2005) Helix I of β-arrestin is involved in postendocytic trafficking but is not required for membrane translocation, receptor binding, and internalization. *Mol. Pharmacol.* **67**, 375–382.

Type 1 angiotensin II receptor-*Renilla* luciferase (AT1R-*Rluc*), and β-arrestin1 and 2 GFP fusion constructs (βarr1-GFP and βarr2-GFP) were created for BRET protein interaction assays. Combinations of AT1R-*Rluc* and β-arrestin-GFP constructs were transfected into COS-7 cells. The COS-7 cell cultures were then activated with 100nM angiotensin II in the presence of 60µM EnduRen™ Live Cell Substrate, and BRET fluorescence readings were taken at 475 and 515nm over a 1-hour period. The authors also describe analysis of helix I mutants of β-arrestin1 and β-arrestin2 in similar β-arrestin-GFP construct BRET studies. Data are displayed as a ratio of fluorescence readings with both constructs compared to fluorescence from the AT1R-*Rluc* construct alone.