Real-time \textit{in vivo} imaging of small laboratory animals is now feasible through the novel use of luciferase reporters and bioluminescence imaging (BLI) technologies, such as IVIS Imaging Systems.\textsuperscript{1} This technology allows real-time monitoring of ongoing biological processes in living animals. The technique is highly sensitive, non-invasive, simple to execute, and can reduce the number of animals used for experimentation since data can be acquired from the same animal over time.

BLI is based on the detection of visible light produced during luciferase-mediated oxidation of the molecular substrate, luciferin, when the enzyme is expressed \textit{in vivo} as a molecular reporter. The luciferase reaction is highly dependent on substrate availability. In fact, studies show that if “exogenously administered luciferin is not abundantly present, light emission might not be a true representation of luciferase activity.”\textsuperscript{2} This constraint can be eliminated with the use of ALZET\textsuperscript{®} Osmotic Pumps, which facilitate steady-state delivery of bioluminescent substrates for extended periods of time. ALZET pumps provide reliable and prolonged substrate delivery, thus eliminating complications of repetitive injections, and ensuring accurate detection of \textit{in vivo} bioluminescence.

When using ALZET pumps with IVIS imaging systems (Caliper Life Sciences, Hopkinton, MA) during BLI studies, researchers should use the BLI-compatible flow moderators in blue or teal color. These were specifically developed by DURECT Corporation to avoid background luminescence caused by the standard (white) flow moderators which may interfere with the real signal from the biological system under study.

Caliper Life Sciences Inc., in collaboration with DURECT, performed a series of studies designed to evaluate the \textit{in vitro} and \textit{in vivo} chemi-luminescence signal emitted from the new flow moderators and compare it to that of the white flow moderators.\textsuperscript{3} These studies indicate that the background signal is significantly reduced with the use of blue and teal flow moderators. Therefore, when using ALZET pumps during BLI studies, researchers should replace the white flow moderators, included in each package of ALZET pumps, with BLI-compatible flow moderators (Refer to Table 1 for a list of flow moderators available).
In Vitro Evaluation of Chemi-luminescence:

The background chemi-luminescence of ALZET pumps containing each of the three different types of flow moderators (white, blue, and teal) was evaluated using the IVIS Imaging System. The open filter was used to measure the maximum chemi-luminescence signal. Figure 1 shows the results of this in vitro evaluation. As seen in Figure 1A, the ALZET pump containing the white flow moderator has significant chemi-luminescence, while the teal and blue flow moderator pumps display no visible chemi-luminescence signal. Graphical representation of light emission (in photons/sec) shows that the white flow moderators display nearly a 100-fold higher chemi-luminescence signal compared to background signal (Fig. 1B). On the other hand, the teal and blue flow moderators only display a 5-fold and 2-fold, respectively, signal above background.

In Vivo Evaluation of Chemi-luminescence:

ALZET Osmotic Pumps (Model 2001) were filled with 240 μl of D-Luciferin solution at a concentration of 50 mg/ml. Nude mice received either subcutaneously implanted ALZET pumps with either colored (blue or teal) or white flow moderators or no pumps (control group). On Day 4, mice were injected subcutaneously with SKOV cells engineered to express luciferase (SKOV3-luc cells). One million cells were injected into the right flank, and 0.5 million cells were injected into the left flank of each mouse. Animals were subjected to BLI at 15, 30, and 120 minutes after cell injection, and luciferase signal intensities were evaluated using image analysis software. Figure 2 shows the in vivo BLI signals from representative animals implanted with pumps containing different colored flow moderators.
As seen in Figure 2, following in vivo BLI of animals implanted with white flow moderator pumps, a significant background chemiluminescence signal is observed at the location of pump implantation, specifically coinciding with the position of the flow moderator. This signal is absent from the images of animals implanted with the teal and blue flow moderator pumps. Moreover, the background signal emitted by the white flow moderator pumps is as strong as the real bioluminescence signal emitted by SKOV3-luc cells. The signal emitted by the white flow moderator pumps can without a doubt be attributed to non-specific chemiluminescence since luciferin oxidation, or real bioluminescence, can only occur in the areas were luciferase-expressing cells were injected (left and right flank). As an additional control of bioluminescence, Figure 3 shows the increase in BLI intensities observed from the areas were animals were injected with 0.5 million cells (line on left) versus 1 million cells (line on the right). In every case, the BLI signal is appreciatively higher in the right flank (1 million cells) compared to the left flank (0.5 million cells).

Fig. 3: Increased intensity of luciferase BLI signal from animals injected with SKOV3-luc cells.
**Recommendations:**

These data indicate that the blue and teal flow moderators of ALZET pumps are preferred for *in vivo* BLI studies using the IVIS imaging systems. These BLI-compatible flow moderators help avoid the background signal associated with the standard (white) flow moderators.

**Research Applications:**

A study by Gross *et al.*, published in Nature Methods, explored the benefits of continuous administration of D-luciferin via ALZET pumps to enable real-time imaging of Iκ kinase (IKK) inhibition in tumor xenografts. The study also looked at the pharmacodynamic properties of the drug candidate PS-1145, a selective IKK inhibitor. Nude mice bearing tumors expressing the IκBα-firefly luciferase fusion reporter (IκBα-Fluc), or unfused luciferase, were subcutaneously implanted with 7-day ALZET pumps containing D-luciferin. Animals were also treated with increasing doses of PS-1145, and imaged with the IVIS imaging system at various time points before and after treatment. The authors found that PS-1145 induced a time-dependent increase in tumor bioluminescence that peaked 8-12 hours after drug administration, followed by a gradual decrease over 32 hours to levels of vehicle treated mice.

The reporter provided continuous, noninvasive monitoring of target-specific IKK activation in real-time. This experimental approach allowed the authors to perform a complete time- and dose-dependent pharmacodynamic analysis of the IKK inhibitor using less than 30 animals. Gross *et al.* also concluded that “the utility of the reporter was further enhanced through innovative use of an implanted micro-osmotic pump for persistent and constant delivery of the bioluminescent substrate D-luciferin… By eliminating constraints of intraperitoneal bolus reinjections of substrate, the implanted pump allowed continuous real-time molecular imaging of reporter activity throughout the time course of a multi-day experiment, while simultaneously allowing rapid analysis of drug action.”

Another study by Abraham *et al.* also describes the use of ALZET pumps for administration of bioluminescence substrates. The study published in the Journal of Neuroscience describes a unique, *in vivo* method to assess circadian rhythms in the olfactory bulb using BLI. This was possible with the use of the *Period1*-luciferase reporter, which was expressed in both intact and suprachiasmatic nuclei-lesioned (SCNX) rats used in the study. For substrate administration, one group of transgenic rats received D-luciferin via intracranial injection; another group was implanted with ALZET pumps (model 2ML1) delivering D-luciferin intraperitoneally. BLI was performed every 4 hours using the IVIS imaging system.

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**Table 1. ALZET BLI-Compatible Flow Moderators**

<table>
<thead>
<tr>
<th>ALZET Flow Moderator‡</th>
<th>Color</th>
<th>Pump Models</th>
<th>Item No.</th>
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<tbody>
<tr>
<td></td>
<td>Blue</td>
<td>100 Series</td>
<td>0002609</td>
</tr>
<tr>
<td></td>
<td>Teal</td>
<td>100 Series</td>
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</tr>
<tr>
<td></td>
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<td>200 Series</td>
<td>0002489</td>
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<td>0002488</td>
</tr>
<tr>
<td></td>
<td>Blue</td>
<td>2ML Series</td>
<td>0002504</td>
</tr>
<tr>
<td></td>
<td>Teal</td>
<td>2ML Series</td>
<td>0002503</td>
</tr>
</tbody>
</table>

*Compatibility evaluated using IVIS Imaging Systems
‡ Sold in quantities of 10 per bag
Animals in the injection protocol required D-luciferin injections every 4 hours for 44 hours, and were subjected to prolonged anesthesia during injection and imaging procedures. In contrast, rats implanted with pumps were anesthetized only briefly every 4 hours for imaging. Imaging results demonstrated rhythmic *Period1* activity in all olfactory bulbs pump-implanted rats, compared to only 70% in the luciferin-injected rats, indicating that “the pump-implantation procedure yielded a higher success rate.” The authors found that a circadian pattern of *Period1* driven activity was present in intact and SCNX rats, providing the first direct evidence of SCN-independent rhythms in the brain.

These studies show that BLI is a powerful tool for studying and monitoring *in vivo* biological processes, including tumor growth. The technique is non-invasive, simple to execute, and can help reduce the number of animals used for experimentation since data can be acquired from the same animal over time. Of equal importance is the appropriate method for substrate delivery. ALZET pumps proved to be an effective method for delivery of luciferin. They provide reliable and prolonged substrate delivery, thus eliminating complications of repetitive injections, and ensuring accurate detection of *in vivo* bioluminescence. For additional information on the use of ALZET pumps for delivery of substrates, including luciferin, please contact ALZET Technical Services.

**References**

1. Caliper Life Sciences Inc., Alameda CA.
3. Study results, images and graphs used with authorization from Caliper Life Sciences Inc.

**Publications mentioning the use of ALZET pumps during BLI studies:**

- P5991 – Rubin et al. Proc Nat Acad Sci 2003;100(23):13513-13518

Contact ALZET Technical Services at 800.692.2990 or alzet@durect.com for instructions on how to adapt ALZET pumps for use during BLI studies.