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PSI SideView™ Imaging Microplate Application Note AN-01

Late-Larval Zebrafish Angiogenesis Assay

SideView™ Imaging of 7 dpf Animals Exposed to VEGF Inhibitor

Objective

The objective of this experiment was to demonstrate the performance of angiogenesis assays in older, more-developed zebrafish larvae using the PSI SideView™ Imaging Microplate technology.

Introduction

There is strong interest in developing zebrafish as a model organism for use in high-content drug discovery assays. Many of the key organs in the zebrafish are best viewed from the side of the animal. Once the swim bladder inflates it is difficult to generate clear side-view images of the animal, especially in multiwell microplate format. Because of this, high-content screening in zebrafish has been limited to embryos and very early larvae.

PSI has developed a novel, SideView imaging microplate (diagrammed in Figure 1) that enables researchers to generate clear side-views of zebrafish larvae.

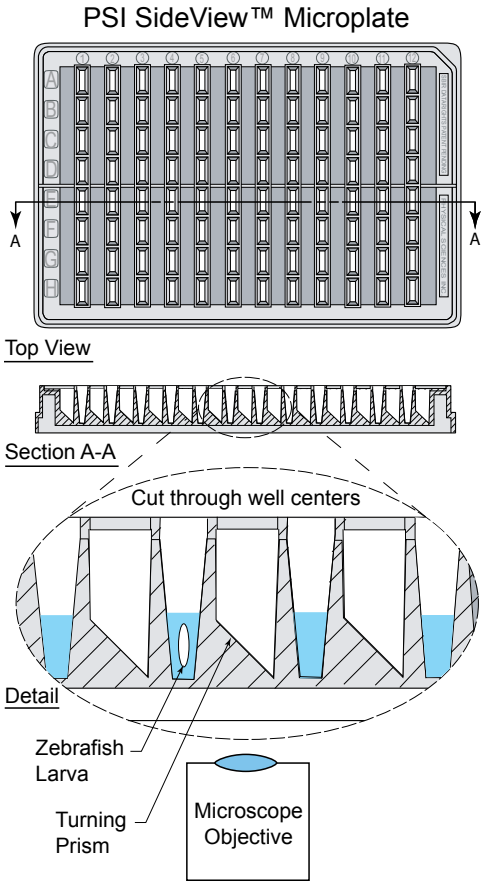

Figure 1.
Top-view schematic representation of the PSI 96 well, SideView microplate technology. With the exception of the rectangular well shapes (top), the microplate dimensions conform to current ANSI/SBS microplate standards and are therefore compatible with standard fluid dispensing and plate handling robotics. The design incorporates right angle viewing prisms that are positioned to enable collection of side-view images of objects in the microwells (middle panels). The objective of an inverted microscope can be positioned under the prism for collection of brightfield and fluorescence images.

Methods

Two VEGF inhibitors, Calbiochem compounds 676475 and 676480, were chosen as model angiogenesis inhibitors. Embryos were loaded into prototype wells and compound was added at approximately 8 hours post-fertilization. Image collection was carried out at 7 days post-fertilization using an inverted microscope equipped with a 2.5x objective and a TE-cooled camera.

Results

Images were collected from unanesthetized animals that were incubated in side-view microwells (Figure 2). The animals were consistently oriented for superior imaging of the vasculature including the Se vessels (Note that the full lengths of the Se vessels are clearly visible in the untreated animal in the side-view array). Both 676475 and 676480 caused defects in vascular patterning most easily noted by incomplete Se development.

Images were analyzed using algorithms developed at PSI. The locations of the animals within the images were first consistently oriented. The images were then subjected to rounds of thresholding and morphological thinning. The location of the DA and the Se branch points were identified, and the Se lengths were determined.

Conclusions

The SideView Microplate enables visualization of the sides of larvae in microwells using an inverted microscope. A vascular image analysis algorithm was also demonstrated.

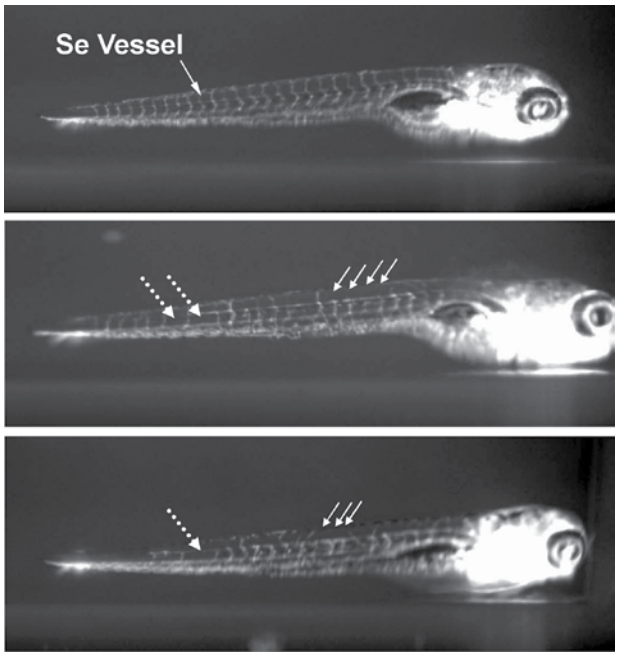

Figure 2.
Images of 7 dpf larvae in SideView™ microwells. The animals were either untreated (top), or were treated with 676480 (middle) or 676475 (lower). Arrows indicate positions of observed vascular defects.

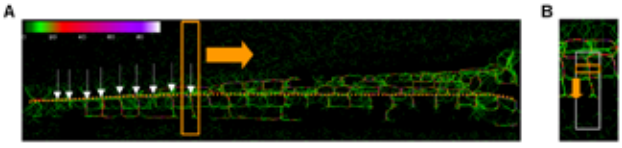

Figure 3.
Schematic representation of the method for determination of Se location (A) and length (B). Orange arrows indicate the direction in which each window is moved along the image. White arrows indicate the locations of each Se start point along the DA (dashed orange line).

Please inquire about your specific application.

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