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Visualizing the Gatekeeper: Evan's Blue Dye-Based Assessment of Blood-Brain Barrier Permeability in Adult Zebrafish

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Abstract

The Blood-Brain Barrier (BBB) is a semi-permeable interface that maintains central nervous system (CNS) homeostasis by regulating the movement of substances into and out of the brain, thereby protecting neural tissue from potentially harmful agents. Disruption of the BBB is a well-established feature in many neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD), where increased permeability contributes to and exacerbates disease progression. Although the adult zebrafish (*Danio rerio*) is increasingly recognized as a valuable model for studying neurodegenerative diseases, standardized methods for evaluating BBB integrity in this species remain limited. This protocol describes a simple, reproducible, and cost-effective approach to assess BBB permeability in adult zebrafish by analysing Evan's Blue (EB) dye extravasation from the neurovasculature into brain tissue. The method combines qualitative visualization with quantitative image analysis of EB distribution to detect BBB disruption. Key steps include proper intraperitoneal

injection of EB dye, confirmation of systemic dye circulation, careful brain dissection, and consistent imaging and greyscale intensity measurement. Positive and negative controls are incorporated to validate dye penetration and support accurate interpretation. Representative results demonstrate greater EB extravasation in Parkinsonian zebrafish brains compared to saline-injected controls, indicating increased BBB permeability. This technique requires minimal specialized equipment and is suitable for laboratories with limited resources. Overall, this protocol offers a practical tool for investigating BBB integrity in adult zebrafish models and can be adapted for diverse applications, including studies of disease mechanisms and the evaluation of therapeutic interventions targeting neurovascular function. © 2025 JoVE Journal of Visualized Experiments.

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Abstract

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