

# Supplementary Materials: Uptake Study in Lysosome-Enriched Fraction: Critical Involvement of Lysosomal Trapping in Quinacrine Uptake but Not Fluorescence-Labeled Verapamil Transport at Blood-Retinal Barrier

## Supplemental information

### *Buffer contents*

#### Phosphate-buffered saline (PBS)

137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4

#### Extracellular fluid (ECF)-buffer

122 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose and 10 mM HEPES, pH 7.4

#### Na<sup>+</sup>-free ECF-buffer

122 mM LiCl, 25 mM KHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose and 10 mM HEPES, pH 7.4

#### ECF-buffer (pH6.4)

122 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose and 10 mM MES, pH 6.4

#### ECF-buffer (pH8.4)

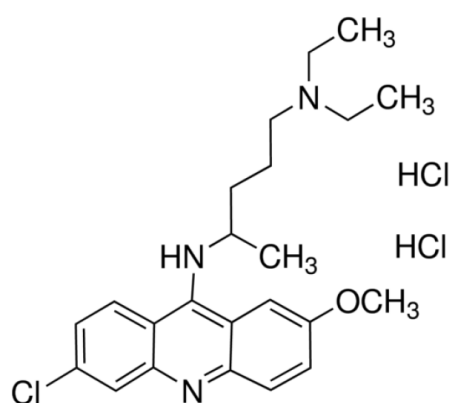
122 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3 mM KCl, 1.4 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 0.4 mM K<sub>2</sub>HPO<sub>4</sub>, 10 mM D-glucose and 10 mM Tris, pH 8.4

### *Cell Culture*

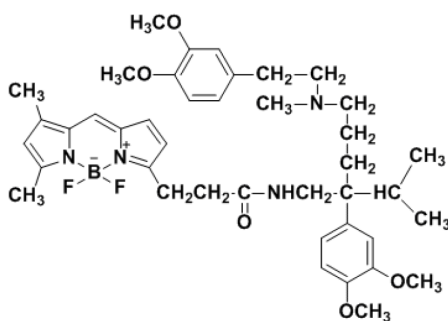
TR-iBRB2 cells (passage number 23-35), a conditionally immortalized rat retinal capillary endothelial cell line, were cultured in Dulbecco's modified Eagle's medium (Nissui Pharmaceuticals, Tokyo, Japan) with 10% fetal bovine serum, 20 mM NaHCO<sub>3</sub>, and antibiotics at 33°C under an atmosphere of 5% CO<sub>2</sub> in air. The optimal growth temperature for TR-iBRB2 cells was set at 33°C because of the expression of temperature-sensitive large T-antigen in TR-iBRB2 cells.

The RPE-J cells (American Type Culture Collection, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (Nissui Pharmaceuticals, Tokyo, Japan) with 4% fetal bovine serum, 20 mM NaHCO<sub>3</sub>, 25 mM D-glucose, 0.1 mM nonessential amino acids, and antibiotics at 33°C under an atmosphere of 5% CO<sub>2</sub> in air. The RPE-J cells were cultured on Not TC-treated culture dishes (Corning, Corning, NY, USA).

## Chemical Structure



Quinacrine.



EverFluor FL Verapamil, (Known as BODIPY® FL Verapamil)



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