

# **Evans Blue**

**Cat**: IE0280

Storage: Powder: 2-8°C, 2 years; Insolvent: -20°C, 6 months; -80°C, 1 year (protect from light)

### Introduction

Evans blue, also known as azo blue, and trypan blue are both cell reactive dyes, which are often used to detect the integrity of cell membranes and cell survival. Living cells cannot be stained blue by Evans blue due to their efflux function, while dead cells will be stained light blue. Therefore, dead cells and living cells can be distinguished under the microscope by this method, but death and necrosis cannot be distinguished.

Evans Blue is a non-permeable dye that can enter the cytoplasm and nucleus and dye them blue when there is damage to the plasma membrane. It can be used to examine cell viability and study the permeability of the blood-brain barrier.

Evans Blue is also an effective L-glutamate uptake inhibitor, through the membrane-bound excitatory amino acid transporter (EAAT).

### **Parameter**

CAS: 314-13-6

Molecular Formula: C<sub>34</sub>H<sub>24</sub>N<sub>6</sub>Na<sub>4</sub>O<sub>14</sub>S<sub>4</sub>

Molecular Weight: 960.81

Appearance: Reddish brown Solid

Solubility: Soluble in Water/DMSO ≥5mg/mL

# 0.2.8.0 NH2 OH NO.2.8.0 Na\* Na\*

## **Protocols** (only for reference)

### (1) Blood-brain barrier permeability

- 1. Take the treated experimental animals (taking mice as an example), inject 0.5%-3% Evans Blue solution (prepared with appropriate buffer) into the tail vein or femoral vein at a ratio of 2-3 mL / kg, and the eyes and tails of the mice should appear blue after injection. The mice were sacrificed after 1 hour of timing, and the target brain tissue was taken. For batch experiments, each mouse should be timing to ensure that each mouse was exposed for 1 hour.
  - Note: The amount of Evans Blue is recommended to be adjusted based on specific experiments.
- 2. Brain tissue was placed in a 1.5 mL centrifuge tube, added with 1 mL of 50% trichloroacetic acid (recommended to use 1 × PBS), quickly homogenized with a tissue homogenizer, and centrifuged at 10000 × g for 20 min.
  - Note: It can also be treated with formamide.
- 3. The supernatant was diluted with anhydrous ethanol four times.
- 4. Take the above solution and measure the absorbance value (OD value) at 620 nm with a spectrophotometer. At the same time, the OD values of standard Evans Blue with different gradients were measured, and the standard curve was drawn. The Evans Blue content of the sample to be tested is calculated according to the standard curve.

### (2) Living cell staining



- 1. Take  $100 \mu L$  re-suspended cells into a conventional centrifuge tube, add  $100 \mu L$  0.5% Evans Blue solution, gently mix and stain (the staining time can be extended appropriately, but not more than 10 min).
- 2. A small amount of stained cells were taken and counted by blood cell counting plate. Usually if you want to quantify more accurately, each cell sample to a small number of 500 cells, counting blue cells and the total number of cells.
- 3. Cell viability = (total number of cells-blue number of cells) total number of cells  $\times$  100 %

# Note

- 1. During cell staining, attention should be paid to the occasional rejection of apoptotic bodies.
- 2. In the blood-brain barrier permeability experiment, the injection volume of Evans Blue solution should be adjusted according to different animals and the weight of animals.
- 3. It is best to use low temperature refrigerated centrifuge for centrifugation.
- 4. For your safety and health, please wear experimental clothes and wear disposable gloves.
- 5. This product is for scientific research only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.

### **Related Literature**

- [1]. Yin T, Du R, Wang Y, Huang J, Ge S, Huang Y, Tan Y, Liu Q, Chen Z, Feng H, Du J, Wang Y, Wang G. Two-stage degradation and novel functional endothelium characteristics of a 3-D printed bioresorbable scaffold. Bioact Mater. 2021 Aug 24;10:378-396. doi: 10.1016/j.bioactmat.2021.08.020. PMID: 34901554; PMCID: PMC8636822. (IF:14.1)
- [2]. Xu H, Wang H, Liang Z, Chen H, Yang D, Tang Z, Dai X. A novel biomineralization-inspired flocculation approach for harvesting high quality microalgal biomass: Dual action of cationic polyelectrolytes and nanosilica. Bioresour Technol. 2023 Nov;388:129739. doi: 10.1016/j.biortech.2023.129739. Epub 2023 Sep 9. PMID: 37696333. (IF:11.4)
- [3]. Liu W, Huang J, He S, Du R, Shi W, Wang Y, Du D, Du Y, Liu Q, Wang Y, Wang G, Yin T. Senescent endothelial cells' response to the degradation of bioresorbable scaffold induces intimal dysfunction accelerating in-stent restensis. Acta Biomater. 2023 Aug;166:266-277. doi: 10.1016/j.actbio.2023.05.028. Epub 2023 May 19. PMID: 37211308. (IF:10.6)

Note: For more literature, please visit the Solarbio official website.

### **Related Products**

ID5110 Trypan Blue

YA0810 Blood cell counting plate

YA0775 Cover glass 24 \* 24mm