Evans Blue Staining Solution (2%)

Cat #: A-CSH374

Size: 100mL

Storage: 2~8°C, avoid light (12 months)

Product Composition

Component	Size	Store at
Evans Blue staining solution (2%)	100ml	2~8°C, and were protected from light

Product Description

Evans Blue, also known as azo blue, has a molecular formula of C34H24N6Na4O14S4 and a molecular weight of 960.80. Its CAS number is 314-13-6. Both Evans Blue and Trypan Blue are cell-viability dyes commonly used to assess cell membrane integrity and cell viability. Live cells are not stained blue, while dead cells are stained pale blue. Live cells, due to their efflux function, cannot be stained by Evans Blue. Therefore, this method can be used to distinguish between dead and live cells under a microscope, but cannot differentiate between cell death and necrosis.

Usage

(I) Blood-brain barrier permeability

1. Take the prepared experimental animals (using mice as an example) and intravenously inject Evans Blue staining solution (2%). The mice's eyes and skin will turn blue. Sacrifice the mice 0.5-1 hour later and collect the desired brain tissue.

2. Place the brain tissue in a 1.5 mL centrifuge tube and add 1 mL of PBS. Quickly homogenize the brain tissue using a tissue homogenizer and centrifuge.

3. Collect the supernatant, add an equal volume of trichloroacetic acid, and incubate at 4°C. Alternatively, the following procedure can be used: collect the supernatant and add acetone in a ratio of 3:7 (supernatant:acetone), and incubate

at room temperature for 24 hours.

4. Centrifuge for 15 minutes.

5. Take the above solution and measure the absorbance at 620 nm using a spectrophotometer (OD value).

Simultaneously measure the OD values of known concentrations of standard Evans Blue to create a standard curve.

Calculate the Evans Blue content in the test sample based on the standard curve.

(II) Live cell staining

1. Take 100 µL of resuspended cells into a regular centrifuge tube and add 100 µL of Evans Blue staining solution (2%).

Gently mix for staining (staining time can be extended, but should not exceed 10 minutes).

2. Take a small amount of stained cells and count using a hemocytometer. Typically, for accurate quantification, at least 500 cells per sample should be counted to determine the number of blue-stained cells and the total cell count.

The formula for calculating cell viability is as follows: Cell Viability = (Total cell count - Number of blue-stained cells) / Total cell count × 100%.

Staining Results

dead cell	blue
living cells	achromatic colour

Notes

1. Evans Blue staining solution (2%) has slight toxicity to the human body, so please handle with care.

2. During cell staining, occasional resistance to staining may occur due to apoptotic bodies.

3. In blood-brain barrier permeability experiments, the injection volume of Evans Blue staining solution (2%) should be adjusted based on different animals and their weights.

4. It is recommended to use a low-temperature centrifuge for centrifugation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.