Evans Blue Staining Solution (0.5%)

Cat #: A-CSH373

Size: 100mL

Storage: Store in dark at 2~8°C (12 months)

Product Description

Evans Blue, also known as azo blue, has a molecular formula of $C_{34}H_{24}N_6Na_4O_{14}S_4$, a molecular weight of 960.80, and a CAS number of 314-13-6. Both Evans Blue and Trypan Blue are cell-viable dyes commonly used to assess the integrity of cell membranes and cell viability. Live cells are not stained blue, while dead cells are stained light blue. Live cells cannot be stained with Evans Blue due to their efflux function. Therefore, this method can be used to differentiate between live and dead cells under a microscope, but it cannot distinguish between cell death and necrosis.

Procedure

Blood-Brain Barrier Permeability

1. Take the processed experimental animals (using mouse as an example) and intravenously inject Evans Blue Staining Solution (0.5%). The mice's eyes and skin will turn blue. After 0.5-1 hour, euthanize the mice and collect the desired brain tissue.

2. Place the brain tissue in a 1.5 ml centrifuge tube and add 1 ml of PBS. Use a tissue homogenizer to quickly homogenize the brain tissue and centrifuge it.

3. Collect the supernatant and add an equal volume of trichloroacetic acid, then incubate at 4°C. Alternatively, the following procedure can be used: collect the supernatant and add acetone in a ratio of supernatant : acetone = 3:7, 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 gradients of standard Evans Blue and plot a standard curve. Calculate the Evans Blue content of the test sample based on the standard curve.

Live Cell Staining

1. Take 100 µl of suspended cells into a regular centrifuge tube and add 100 µl of Evans Blue Staining Solution (0.5%). 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 them using a hemocytometer. Typically, for accurate quantification, at least 500 cells per cell sample should be counted, counting the number of blue cells and the total number of cells.

The formula for calculating cell viability is as follows:

$$\text{Cell Viability} = (\text{Total Cell Count} - \text{Blue Cell Count}) / \text{Total Cell Count} \times 100\%$$

Staining Results

Dead cells	Blue
Live cells	Colourless

Notes

1. The Evans Blue staining solution (0.5%) has slight toxicity to the human body, so please take precautions.
2. During cell staining, be aware that apoptotic bodies may occasionally exhibit resistance to staining.
3. In blood-brain barrier permeability experiments, the injection volume of 0.5% Evans Blue staining solution should be adjusted according to different animals and their weights.
4. It is preferable to use a low-temperature centrifuge for the centrifugation process.
5. For your safety and health, please wear lab coats and disposable gloves when performing the procedure.

Disclaimer

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