Evans Blue Staining Solution (1%)

E1507838

Storage: 2-8℃. Store in the dark.

Introduction:

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 960.81, and a CAS number of 314-13-6. As a commonly used azo dye preparation, Evans Blue has a molecular weight similar to that of plasma albumin and exhibits high affinity with plasma albumin in the blood. Therefore, it is often used in neuroscience research for tracing and observing the integrity of the blood-brain barrier (BBB). It is also applied in cell staining to distinguish between live and dead cells, and can be used for blood volume determination. Clinically, Evans Blue is used as a drug to measure plasma and blood volume, and also for the positioning of arterial cannulation. Under normal circumstances, plasma albumin cannot penetrate the BBB; thus, if the BBB of the nervous system remains intact after staining, Evans Blue bound to plasma albumin will not cause staining of the nervous system. On the contrary, if the BBB of the nervous system is damaged, Evans Blue can enter the nervous system and stain it. Evans Blue shows strong absorption peaks at fluorescence wavelengths of 470 nm and 540 nm, and a weak peak at 680 nm, and can be detected by chemical dialysis and colorimetry.

Both Evans Blue and trypan blue are cell viability dyes, commonly used to detect the integrity of cell membranes and cell viability. Live cells will not be stained blue, while dead cells will be stained light blue. After Evans Blue staining, the cell survival rate can be quantified relatively accurately by direct counting under a microscope or counting after microscopic photography. The most commonly used concentration is 0.5%. Live cells cannot be stained by Evans Blue due to their efflux function, so this method can be used to distinguish dead cells from live cells under a microscope, but cannot distinguish between apoptosis and necrosis. Evans Blue Staining Solution (1%) is mainly composed of Evans Blue and phosphate solution. This reagent is for research use only and not for clinical diagnosis or other purposes.

Materials to Be Prepared by Users:

1. Syringes, tissue homogenizers.
2. PBS (Phosphate-Buffered Saline), trichloroacetic acid, or acetone.

Operating Procedures (for Reference Only):

Before use, dilute the Evans Blue Staining Solution (1%) with PBS to prepare Evans Blue Staining Solution (0.5%) (Product No:M1507837).

(1) Blood-Brain Barrier Permeability Test:

1. Take the treated experimental animals (mice as an example), and inject Evans Blue Stain (0.5%) via the tail vein or femoral vein at a dose of 2-3 ml/kg. Within a few seconds to 1

minute, the mice's eyes and skin will turn blue. Sacrifice the mice 0.5-1 hour later and collect the target brain tissue.

2. Place the brain tissue in a 1.5 ml centrifuge tube, add 1 ml of PBS, and quickly homogenize the brain tissue using a tissue homogenizer. Centrifuge at 1000×g for 15 minutes.
3. Collect the supernatant, add an equal volume of trichloroacetic acid, and incubate at 4°C for 18-24 hours. Alternatively, the following operation can be adopted: collect the supernatant, add acetone at a ratio of supernatant:acetone = 3:7, and incubate at room temperature for 24 hours.
4. Centrifuge at 1000×g for 20-30 minutes or at 2000×g for 15 minutes.
5. Take 1-2 ml of the above solution, measure the absorbance (OD value) at 620 nm using a spectrophotometer. Meanwhile, determine the OD values of standard Evans Blue solutions with different gradients, draw a standard curve, and calculate the Evans Blue content in the sample to be tested based on the standard curve.

(2) Live Cell Staining:

1. Take 100 μl of resuspended cells into a conventional 1.5 ml or 0.5 ml centrifuge tube, add 100 μl of Evans Blue Staining Solution (0.5%) (Product No.: M1507837), mix gently, and stain for 3 minutes (the staining time can be appropriately extended but should not exceed 10 minutes).
2. Aspirate a small amount of stained cells and count them using a hemocytometer. For relatively accurate quantification, at least 500 cells should be counted for each cell sample, and the number of blue cells and the total number of cells should be recorded. The calculation formula is as follows:
   Cell Survival Rate = [(Total Number of Cells - Number of Blue Cells) / Total Number of Cells] × 100%

(3) Seed Staining:

1. Use a blade to make cross-sections and accurate longitudinal sections along the center of the seed embryo, then immerse the sections in the staining solution for 3-5 minutes.
2. Soak the stained sections in distilled water for 20-60 minutes, depending on the degree of decolorization.

Precautions:

1. Evans Blue Staining Solution (1%) has slight toxicity to the human body; please take careful protective measures.
2. During cell staining, note that apoptotic bodies may occasionally exhibit dye exclusion (i.e., not be stained).
3. In the blood-brain barrier permeability test, the injection volume of Evans Blue Staining Solution (0.5%) (Product No.: M1507837) should be adjusted according to the type and weight of the experimental animals.
4. It is recommended to use a low-temperature refrigerated centrifuge for centrifugation.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.