Evans Blue Staining Solution (0.1%)

Cat. No.: E1507841 | Pack size: 100 mL | Storage: Store at 2-8°C, Protected from light

Overview

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 high affinity with plasma albumin in the blood. Therefore, it is often used in neuroscience research to trace and observe the integrity of the blood-brain barrier (BBB). It is also used for cell staining to distinguish between live and dead cells, and 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 blood-brain barrier. Thus, if the BBB in the nervous system remains intact after staining, Evans Blue bound to plasma albumin will not stain the nervous system. On the contrary, if the BBB 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 (0.1%) 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)

(1) Blood-Brain Barrier Permeability Test

1. Take the treated experimental animals (mice as an example), and inject Evans Blue Staining Solution (0.1%) 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 (0.1%), 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.

(4) IFA Counterstaining for Paraffin Sections

1. Perform routine slide baking and dewaxing.

2. Incubate with the secondary antibody containing Evans Blue at a specific concentration (0.1%) for counterstaining, lasting 30 minutes.

3. Mount the sections with buffered glycerol and observe under a microscope.

(5) Viable Cell Detection in Intact Plant Leaves

1. Take the leaves treated with stress, wash them thoroughly with pure water, dry them, and soak them in 0.25% (W/V) Evans Blue solution for 24 hours.

2. Rinse the surface of the leaves with clean water to remove the blue staining solution, blot the surface moisture, and immerse the leaves in boiling absolute ethanol:glycerol (9:1) solution for 30 minutes. Continue until chlorophyll is removed and the leaves turn pale, then take photos to record the leaves with blue spots.

3. Cut the leaves into pieces, place them in 4 mL of 1% SDS solution for extraction over 3 days. Use leaves fully stained blue by Evans Blue (after boiling in water for 15-20 minutes) as the control. Measure the absorbance of the extract at 600 nm.

Safety & Precautions

- 1. Evans Blue Staining Solution (0.1%) 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 (0.1%) 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.
- 6. Wear a lab coat, disposable gloves and eye protection during handling.
- 7. Use only under an approved research protocol and in accordance with applicable institutional guidelines.

Key Features

- Supplied as BioReagent, Biological Stain, for microscopy, 0.1% grade material for research workflows.
- Designed for use in Cell analysis, Cell Staining, Live/Dead Cell Assay applications.
- Available pack size: 100 mL.
- Storage condition: Store at 2-8°C, Protected from light.
- Related name(s): Evans Blue Staining Solution.
- For research use only; not intended for clinical diagnosis, treatment, food, or household applications.

Product Information

Item	Description
Cat. No.	E1507841
Product Name	Evans Blue Staining Solution (0.1%)
Specifications & Purity	BioReagent, Biological Stain, for microscopy, 0.1%
Synonyms	Evans Blue Staining Solution
Pack Size	100 mL
Storage	Store at 2-8°C, Protected from light
Stability & Storage	Store at 2-8°C long term (12 months). Store in the dark.
Shipped In	Wet ice. This product requires cold chain shipping. Ground and other economy services are not available.
Recommended Application	Cell analysis, Cell Staining, Live/Dead Cell Assay
CAS	314-13-6
Molecular Weight	960.81
EC Number	206-242-5
Beilstein Registry Number	16(2)259
Citations	125

Names & Identifiers

Identifier	Value
Isomeric SMILES	CC1=C(C=CC(=C1)C2=CC(=C(C=C2)N=NC3=C(C4=C(C=C3)C(=CC(=C4N)S(=O)(=O)[O-])S(=O)(=O) [O-])O)C)N=NC5=C(C6=C(C=C5)C(=CC(=C6N)S(=O)(=O)[O-])S(=O)(=O)[O-])O.[Na+].[Na+].[Na+].[Na+]
WGK Germany	3
RTECS	QJ6440000
Molecular Weight	960.81
Beilstein	16(2)259
Reaxy-Rn	7110511

Identifier	Value
Reaxys-	https://www.reaxys.com/reaxys/secured/hopinto.do?context=S&query=IDE.XRN=7110511&ln= RN_link_address

Chemical and Physical Properties

Property	Value
Sensitivity	Light-sensitive

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Personal protective equipment	Lab coat, disposable gloves, eye protection	Safe handling and contamination control
Microscope or imaging system	Configured for the intended staining or observation workflow	Signal observation and documentation
Pipettes and tips	Clean disposable tips	Reagent and sample handling
PBS, distilled water or normal saline	As required by the source procedure	Washing, dilution or sample preparation

Quality Control

QC Item	Method	Acceptance Criteria
Visual inspection on receipt	Confirm that the container is intact and the label, lot number, pack size and expiry information are legible.	No leakage, visible damage or labeling inconsistency.
Product identity and specification	Check the product label and lot-specific COA against the product information in this manual.	Product name, Cat. No., grade/purity and storage condition match the ordered product.
Appearance / usability	Inspect before use according to the source precautions and applicable laboratory SOPs.	No abnormal precipitation, discoloration, contamination or degradation that would interfere with the intended assay.

QC Item	Method	Acceptance Criteria
Staining performance	Evaluate positive/negative or expected sample response under the recommended observation conditions.	Expected staining pattern is observed with acceptable background.

Troubleshooting

Issue	Possible Causes	Corrective Action
Weak or uneven staining / signal	Insufficient staining time, incomplete sample coverage, quenching, expired or improperly stored reagent	Ensure complete sample coverage, follow the source procedure, protect fluorescent dyes from light, and use fresh or properly stored reagent.
High background	Excess staining solution, inadequate washing, overly long staining time	Optimize staining duration, wash as recommended, and avoid over-staining.
Signal fades quickly	Photobleaching or repeated freeze-thaw cycles	Protect from light, minimize exposure during observation, and avoid repeated freeze-thaw cycles where applicable.

Recommended Applications

Cell analysis, Cell Staining, Live/Dead Cell Assay.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, please contact Aladdin Scientific through the offices below.

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, food, cosmetic, or household applications unless specifically authorized by applicable documentation.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared or FDA-approved diagnostic product.
- Where any product or component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), users must follow the current SDS, product label and local regulations.
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