伊文斯蓝染色液(0.5%)

货号： G1810
规格： 100mL/500mL
保存： 室温，避光保存，有效期 1 年。

产品介绍：

伊文斯蓝(Evans Blue)又称偶氮蓝。伊文斯蓝与台盼蓝都是细胞活性染料，常用于检测细胞膜的完整性和细胞是否存活。活细胞因有外排功能而无法被伊文斯蓝染成蓝色，而死细胞会被染成淡蓝色。因此可以通过此方法在显微镜下区分死细胞与活细胞，但无法区分死亡与坏死。

操作步骤：(*仅供参考*)

(一) 血脑屏障通透性

1. 取处理后的实验动物(以小鼠为例)，经尾静脉或股静脉按照 2-3ml/kg 的比例注射伊文斯蓝染色液(0.5%)，小鼠眼睛、尾巴应在注射后 10s 内出现蓝色，1h 后耳朵爪子应变蓝。计时 1h 后处死小鼠，取目的脑组织，如批量实验需分别计时保证每只小鼠都暴露 1h。
2. 脑组织置于 1.5mL 离心管中，加入 1mL 用 1×PBS 稀释的 50%三氯乙酸， 迅速用组织匀浆器将脑组织制成匀浆, 10000×g 离心 20min。
3. 取上清用无水乙醇四倍稀释。
4. 取上述溶液，用分光光度计测 620nm 处吸光值(OD 值)。同时测定已知不同梯度的标准伊文斯蓝的 OD 值，绘制标准曲线。根据标准曲线计算出待测样品的伊文斯蓝含量。

(二) 活细胞染色

1. 取 100μl 重悬细胞到常规离心管内，加入 100μl 0.5%伊文斯蓝染色液轻轻混匀染色(染色时间可适当延长，但不宜超过 10min)。
2. 吸取少量经过染色后的细胞，用血细胞计数板计数。通常如果要比较精确地进行定量，每个细胞样品至少数 500 个细胞，数出蓝色细胞和细胞总数。细胞存活率计算公式如下：
3. 细胞存活率＝(细胞总数－蓝色细胞数)/细胞总数×100%

注意事项：

1. 伊文斯蓝染色液(0.5%)对人体有轻微毒性，请小心防护。
2. 细胞染色时，注意凋亡小体偶尔也有拒染现象。
3. 血脑屏障通透性实验中，染色试剂注射量应根据不同动物以及动物的重量调整。
4. 最好采用低温冷冻离心机进行离心。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Evan's Blue Stain Solution, 0.5%

Cat: G1810
Size: 100mL/500mL
Storage: RT, avoid light, valid for 1 year.

Introduction

Evans Blue and trypan blue are both cell reactive dyes, which are commonly used to detect the integrity of cell membrane and cell survival. Living cells can not be dyed blue by Evans Blue because of their efflux function, while dead cells can be dyed light blue. Therefore, dead cells and living cells can be distinguished under microscope by this method, but death and necrosis cannot be distinguished.

Protocols(*for reference only*)

Blood-brain barrier permeability

1. Take the treated experimental animals (taking mice as an example) and inject Evan's Blue Stain Solution, 0.5% through the tail vein or femoral vein at the ratio of 2-3ml / kg. The eyes and tails of mice should appear blue within 10s after injection, and the ears and claws should become blue after 1h. The mice were killed after 1h, and the target brain tissue was taken. If the batch experiment was conducted, the time should be measured separately to ensure that each mouse was exposed for 1H.
2. Brain tissue was placed in a 1.5ml centrifuge tube and 1ml of 1 × 50% trichloroacetic acid diluted in PBS, brain tissue was rapidly homogenized with a tissue homogenizer, 10000 × G for 20min.
3. The supernatant was diluted four times with absolute ethanol.
4. Take the above solution and measure the absorbance value (OD value) at 620nm with a spectrophotometer. At the same time, the OD value of standard Evans blue with different gradients was measured and the standard curve was drawn. Calculate the Evans blue content of the sample to be measured according to the standard curve.

Living cells staining

1. Take 100μl of re-suspension cells into the conventional centrifuge tube, add 100μl Evan's Blue Stain Solution, 0.5% into the tube and mix it gently for staining (the staining time can be appropriately prolonged, but not more than 10 minutes).
2. Take a small number of stained cells and count them with a blood cell counting board. Usually, if you want to make a more accurate quantitative analysis, count at least 500 cells for each cell sample and count the number of blue cells and the total number of cells. The cell survival rate is calculated as follows:
   Cell survival rate = (total number of cells - number of blue cells) / total number of cells × 100%

Note

1. Evan's Blue Stain Solution, 0.5% is slightly toxic to human body, pay attention to self protection.
2. When staining cells, note that apoptotic bodies occasionally reject staining.
3. In the blood-brain barrier permeability experiment, the injection amount of Evan's Blue Stain Solution, 0.5% should be adjusted according to the weight of different animals and animals.
4. It is best to use a cryocentrifuger for centrifugation.
5. For your safety and health, please wear experimental clothes and disposable gloves.