# 阿利新蓝茜素红双染实验报告

## 一、实验器材及试剂

1、实验器材

| 名称 | 厂家 | 型号 |
|---|---|---|
| 解剖镊 | | |
| 解剖剪 | | |
| 照相机 | 佳能 | EOS70D |
| 培养皿 | | |
| 标本瓶 | Wanwu | G6008-40ML |

2、主要实验试剂

| 试剂名称 | 厂家 | 货号 |
|---|---|---|
| 阿利新蓝 | SIGMA | A5268 |
| 茜素红 | 西亚 | |
| 冰醋酸 | 国药集团化学试剂有限公司 | 10000218 |
| 乙醇 | 国药集团化学试剂有限公司 | 100092683 |
| 甘油 | 国药集团化学试剂有限公司 | 10010618 |
| KOH | 国药集团化学试剂有限公司 | 10017018 |
| 丙酮 | 国药集团化学试剂有限公司 | 10000428 |

## 二、试剂配制

1、阿利新蓝 8GS：0.3g 阿利新蓝粉剂以 70%的乙醇为溶剂定容至 100ml。

2、茜素红：0.1g 茜素红粉剂以 95%的乙醇为溶剂定容至 100ml。

3、阿利新蓝-茜素红染液：将 0.3%阿利新蓝：0.1%茜素红：冰醋酸：70%乙醇按照 1：1：1：17 的比例充分混合。

4、透明液：20%甘油：1% KOH 按 1：4 混合。

5、保存液：50%甘油。

6、1%KOH：1gKOH 粉剂溶于 100ml 纯水中完全溶解。

## 三、实验步骤

1、样品处理：取材后先用 95%的乙醇固定 5 天或更长时间的，固定时间不要超过 3 个月。

2、骨架的分离与固定：丙酮处理 2-7 天（一般孕 13-16 天直接处理 2 天，孕 16 天以上的用两只尖头镊子剥下胎鼠的皮及内脏后处理 7 天），丙酮完全浸没组织，处理时间依组织大小可适当延长。

3、染色：对于孕期 13-16 天的胎鼠：用镊子将样品取出，入装有 30ml 阿利新蓝-茜素红染液的样本瓶中染色 4h 后，即可入 1%KOH 透明 1 天（具体以骨架透明为主），对于孕期 16 天以上的胎鼠：用镊子将样品取出，入装有 40ml 阿利新蓝-茜素红染液的样本瓶中染色 2-3 天后观察爪子及舌头处是否为砖红色，边染边观察整体是否偏蓝，若开始上蓝色就取出纯水洗，即可入 1%KOH 透明 2 天（具体以骨架透明为主）。

4、透明及拍照：取出样品，入 20%甘油中透明 1 周，直至骨架和肌肉都清晰为主，最后转入 50%甘油中拍照。

5、保存：50%甘油中保存样品。

**四、结果判读**

1、小鼠骨架清晰透明，骨架呈现紫红色，角质化的皮肤和肌肉呈蓝色，对比清晰。

**五、注意事项**

1、脱水和丙酮处理时间不能太久，不染样品会出现染色不均，尤其是爪子和尾巴。

2、染色时间要严格控制，染得过久，骨架整体偏蓝，染得过浅骨架容易褪色。

3、1%KOH 处理不能过久，不然骨架容易崩解。

**六、图片**

# Alcian blue and alizarin red staining experimental report

## 1. Experimental equipment and reagents

1.1 Experimental equipment

| Name | Manufactor | Model |
|---|---|---|
| Anatomic forceps | | |
| Dissecting scissors | | |
| Camera | Canon | EOS70D |
| Culture dish | | |
| Specimen bottle | Wanwu | G6008-40ML |

1.2 Main experimental reagents

| Reagent name | Manufactor | Article number |
|---|---|---|
| Alcian Blue | Sigma | A5268 |
| Alizarin red | West Asia | |
| Glacial acetic acid | Sinopharm Group Chemical Reagent Co. LtD | 10000218 |
| Ethanol | Sinopharm Group Chemical Reagent Co. LtD | 100092683 |
| Glycerol | Sinopharm Group Chemical Reagent Co. LtD | 10010618 |
| Potassium hydroxide | Sinopharm Group Chemical Reagent Co. LtD | 10017018 |
| Acetone | Sinopharm Group Chemical Reagent Co. LtD | 10000428 |

## 2. Reagent preparation

2.1 Alcian blue 8GS: 0.3 g alcian blue powder was diluted to 100 mL with 70% ethanol as solvent.

2.2 Alizarin red: 0.1g alizarin red powder was diluted to 100mL with 95% ethanol.

2.3 Alcian blue-alizarin red dye solution: mix 0.3% alcian blue: 0.1% alizarin red: glacial acetic acid: 70% ethanol in the ratio of 1:1:1:17.

2.4 1% glycerin:1% KOH in the ratio of 1:4

2.5 Preservation solution: 50% glycerin.

2.6 1% KOH: 1 g KOH powder is dissolved in 100 mL pure water.

**3. Experimental step**

3.1 Separation and fixation of skeletons: acetone treatment for 2~7 days （usually directly treated for 2 days during 13~16 days of gestation, and the skin and viscera of fetal rats were peeled off with two tweezers after pregnancy for more than 16 days）, the tissues were completely iersed in acetone, and the treatment time could be appropriately prolonged according to the tissue size.

3.2 Acetone treatment for 2~7 days （usually direct treatment for 2 days at 13~16 days of pregnancy, and 7 days after peeling the skin and viscera of fetal rats with two tweezers）, the tissue was completely iersed in acetone, and the treatment time can be appropriately prolonged according to the size of the tissue.

3.3 Take out the sample, put it in 20% glycerol and transparent for 1 week, until the skeleton and muscle are clear, and finally transfer to 50% glycerol to take pictures.

3.4 The sample was preserved in 50% glycerol.

**4. The results were as follows**

4.1 The skeleton of mice is clear and transparent, the skeleton is purplish red, and the keratinized skin and muscle are blue, and the contrast is clear.

**5. Precautions**

5.1 Dehydration and acetone treatment time should not be too long, samples will appear uneven dyeing, especially claws and tail.

5.2 The dyeing time should be strictly controlled. If the dyeing time is too long, the whole skeleton will turn blue, if it is too light, the skeleton will fade easily.

5.3 1% KOH treatment should not be too long, otherwise the skeleton will disintegrate easily.

**6. Pictures**