

# 茜素红 S 染色定量检测试剂盒(CPC 法)

V02

货号: G3283

**规格:** 2×20mL/2×100mL

**保存:** 2-8℃, 避光保存, 有效期6个月。

## 产品组成:

名称	2×20mL	2×100mL	保存
试剂(A): 染色液	20mL	100mL	2-8℃, 避光
试剂(B): 标准液	1mL	5mL	2-8℃, 避光
试剂(C): 洗脱液	20mL	100mL	RT

## 产品介绍:

茜素红 S 属一种蒽醌类衍生物,是茜素磺酸钠盐,它能与碳酸钙或磷酸钙中的钙盐螯合形成橙红色复合物,适用于组织切片和培养细胞的钙盐沉积染色。为了方便分析钙盐含量和浓度,茜素红 S 的染色可以使用 CPC 试剂完全洗脱,洗脱下来的溶液置于 96 孔板内,通过测量 560nm 下的 OD 值对钙盐含量进行半定量分析。

该试剂盒由染色液、标准液和洗脱液组成,适用于细胞和组织样本中钙盐的茜素红 S 染色、提取和测定实验。

## 操作步骤: (仅供参考)

试剂(C): 洗脱液在低温环境下容易析出结晶,建议单独置于室温环境下存放,若观察有析出,可置于37℃水浴或60℃ 烘箱加热溶解后使用。

- 1. 对于组织切片:石蜡切片厚度 4μm,常规脱蜡至水;对于培养细胞:细胞使用 4%多聚甲醛或 10%中性福尔马林固定液室温固定 15-30min,蒸馏水洗 2 次,每次 3min 去除多余固定液。
- 2. 滴加试剂(A):染色液进行茜素红 S 染色 3-5min。然后蒸馏水快速洗去多余染色液。
- 3. 对于组织切片,滴加 400ul 试剂(C): 洗脱液室温孵育切片 15-30min;对于细胞样本,六孔板建议每孔加入 1ml 试剂(C): 洗脱液室温孵育细胞 15-30min,直至茜素红 S 着色完全洗脱。
- 4. 用离心管收集洗脱下来的茜素红 S 溶液并充分摇匀。 置于 96 孔板内,每孔添加 100uL,共三个重复 孔,在酶标仪 560nm 波长下测定 OD 值。

### 标曲配制

将试剂(B):标准液先用试剂(C): CPC 洗脱液稀释 20 倍得到浓度为 0.5g/L 的标准溶液,然后依次进行倍比稀释得到 0.25g/L、0.125g/L、0.0625g/L、0.03125g/L、0.015625g/L 的标准溶液,以试剂(C): 洗脱液为空白对照,置于 96 孔板内,每孔添加 100uL,在酶标仪 560nm 波长下测定 OD 值,整理数据制定标曲。

### 测定结果:

将待检样本所得 OD 值代入标曲线性方程,得到待检样本对应茜素红 S 浓度,从而半定量钙盐含量。

### 注意事项:

- 1. 同一批样本测定实验尽量在同一天进行,实验间隔超过三天的样本建议重新制定标曲。
- 2. 为了您的安全和健康,请穿实验服并戴一次性手套操作。

## 相关产品:

G1450 茜素红S染色液(0.2%,pH8.3)

G1452 茜素红S染色液(1%,pH4.2)

G1453 茜素红S染色提取试剂(CPC, 10%)

G3280 钙盐染色试剂盒(改良茜素红S法)

G3281 钙盐染色试剂盒(茜素红S法)



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## Alizarin Red S Staining Quantitative Detection Kit (CPC Method)

Cat: G3283

**Size:** 2×20mL/2×100mL

Storage: 2-8°C, avoid Light, valid for 6 months.

### **Kit Components**

Reagent	2×20mL	2×100mL	Storage
Reagent(A): Stain Solution	20mL	100mL	2-8°C, avoid Light
Reagent(B): Standard Solution	1mL	5mL	2-8°C, avoid Light
Reagent(C): Elution Solution	20mL	100mL	RT

### Introduction

Alizarin Red S is an anthraquinone derivative, which is a sodium salt of Alizarin Sulfonate. It can chelate with calcium salts in calcium carbonate or calcium phosphate to form an orange red complex, suitable for tissue sectioning and calcium salt deposition staining of cultured cells. For the convenience of analyzing calcium salt content and concentration, Alizarin Red S staining can be completely eluted with CPC reagent. The eluted solution is placed in a 96 well plate, and the calcium salt content is semi quantitatively analyzed by measuring the OD value at 560nm.

This reagent kit consists of Stain Solution, Standard Solution, and Elution Solution, and it is suitable for Alizarin Red S staining, extraction and determination experiments of calcium salts in cell and tissue samples.

## **Protocol**(*for reference only*)

Reagent(C): Elution Solution is prone to crystallization in low temperature environments. It is recommended to store it separately at room temperature. If precipitation is observed, it can be dissolved in a 37 °C water bath or a 60 °C oven after heating.

- 1. For tissue sections: paraffin section thickness 4 μm. Conventional dewaxing to water. For cultured cells, they are fixed at room temperature for 15-30 minutes using 4% paraformaldehyde or 10% neutral formalin fixative. Rinse with distilled water twice for 3min each.
- 2. Add Reagent(A): Stain Solution and stain for 3-5 minutes. Then quickly wash off excess staining solution.
- 3. For tissue sections, add 400ul Reagent(C): Elution Solution onto the section and incubate at room temperature for 15-30 minutes; for cultured cells, six well plate add 1ml Reagent(C): Elution Solution and incubate at room temperature for 15-30 minutes, until the Alizarin Red S staining is completely washed off.
- 4. Collect the eluted Alizarin Red S solution using a centrifuge tube and shake well. Place in a 96 well plate, add 100uL to each well, with a total of three repeated wells, and measure the OD value at a wavelength of 560nm on an enzyme-linked immunosorbent assay (ELISA) reader.

## Standard curve preparation

Dilute Reagent(B): Standard Solution by 20 times with Reagent(C): Elution Solution to prepare standard solution of 0.5g/L), and then perform multiple dilution in sequence to obtain standard solutions of 0.25g/L, 0.125g/L, 0.0625g/L, 0.03125g/L and 0.015625g/L. Use Reagent(C): Elution Solution as the blank control. Place in a 96 well plate, add 100uL to each well, measure the OD value at a wavelength of 560nm on the enzyme-linked immunosorbent assay (ELISA), and organize the data to develop a standard curve.

#### Result

Substitute the OD value obtained from the test sample into the standard curve linear equation to obtain the corresponding Alizarin Red S concentration of the test sample, thereby semi quantitatively determining the calcium salt content.

### Note

- 1. The determination experiment of the same batch of samples should be conducted on the same day as much as possible. For samples with an interval of more than three days, it is best to redefine the standard curve.
- 2. For your safety and health, please wear laboratory clothes and disposable gloves when operating.

### Related products

G1450 Alizarin Red S Solution, 0.2%, pH8.3

G1452 Alizarin Red S Solution, 1%, pH4.2

G1453 Alizarin Red S Staining Extraction Solution (CPC, 10%)

G3280 Calcium Stain Kit (Modified Alizarin Red S Method)

G3281 Calcium Stain Kit (Alizarin Red S Method)

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