

## 新型隐球菌染色试剂盒（Steedman-Lison 法）

货号：G3205

规格：2×50mL

保存：室温，避光保存，有效期 1 年

### 产品组成：

名称	2×50mL	保存
试剂(A)：阿利新蓝染色液	50mL	室温，避光
试剂(B)：复红染色液	50mL	室温，避光

### 产品介绍：

新型隐球菌常采用墨汁染色检测法进行检测，尤其在疑为中枢神经系统隐球菌感染的脑脊液标本检测时。但在实际应用中，墨汁染色法受杂质及细胞成分干扰大，检出率低，特别在中枢感染的初期及恢复期，隐球菌数量较少，易漏检。本试剂盒采用阿利新蓝法，染色特异性高，着色清晰，能有效提高检出率。

### 操作步骤：（仅供参考）

#### 对于石蜡切片：

1. 取新鲜组织进行石蜡包埋和切片，常规脱蜡至水，蒸馏水洗两次，每次 2min。
2. 阿利新蓝染色液滴染或浸染 10-20min，蒸馏水稍洗 5-10s。
3. 复红染色液滴染或浸染 5-10min，蒸馏水稍洗 5-10s。
4. 脱水透明：95%乙醇 10s，100%乙醇I 10s，100%乙醇II 1min，二甲苯I 1min，二甲苯II 1min。
5. 中性树胶封片。

#### 对于菌液涂片

1. 挑取菌落或离心富集的菌液用适当 1×PBS 稀释，混匀静置 10-20min 充分分散。
2. 吸取 5-10ul 充分分散的菌液涂布在载玻片上，静置 5min 后倾去多余未附着菌液或 37℃加热台晾干。
3. 晾干后滴加 200ul 甲醇或 95%乙醇进行固定，固定 3min 后倾去多余液体，晾干。
4. 阿利新蓝染色液滴染或浸染 10-20min，蒸馏水稍洗 5-10s。
5. 复红染色液滴染或浸染 5-10min，蒸馏水稍洗 5-10s。
6. 直接晾干镜检或晾干后中性树胶封片镜检。

### 染色结果：

新型隐球菌荚膜	蓝色
细胞核、菌体	红色
酸性粘液质和软骨	蓝色

### 注意事项：

1. 初次染色建议取 1-2 个样本进行预实验。
2. 对于特殊细胞，如果细胞沉淀不充分，可以适当提高离心力或延长离心时间。





# Cryptococcus Neoformans Stain Kit (Steedman-Lison Method)

**Cat:** G3205

**Size:** 2×50mL

**Storage:** RT, avoid light, valid for 1 year.

## Kit Components

Reagent	2×50mL	Storage
Reagent(A): Alcian Blue Staining Solution	50mL	RT, avoid light
Reagent(B): Red Staining Solution	50mL	RT, avoid light

## Introduction

The staining of *Cryptococcus neoformans* is often detected by ink staining, especially in cerebrospinal fluid samples suspected of cryptococcus infection of the central nervous system. However, in practical application, the ink staining method is greatly disturbed by impurities and cell components, and the detection rate is low. Especially in the early stage and recovery period of central infection, the number of *Cryptococcus* is small, which is easy to miss detection.

This kit adopts alcian blue method, which has high staining specificity and clear staining effect, and can effectively improve the detection rate.

## Protocols(for reference only)

### For Paraffin section

1. Pick fresh tissue and embed to cut into paraffin sections, dewax to water, washed twice with distilled water, 2min each time.
2. Dye with Alcian Blue Staining Solution for 10-20min, and wash slightly with distilled water for 5-10s.
3. Redyeing with Red Staining Solution for 5-10min and slightly washing with distilled water for 5-10s.
4. Dehydration and transparency: 95% ethanol for 10s, 100% ethanol for 1min, xylene for 2min.
5. Neutral gum seal.

### For Bacterial smear

1. Pick up colonies or centrifugally enriched bacterial solution and use appropriate 1×PBS, mix well and let stand for 10-20min to fully disperse.
2. Absorb 5-10ul of fully dispersed bacterial solution and apply it on the slide. After standing for 5min, dump the excess unattached bacterial solution or dry it on a 37 °C heating table.
3. After drying, add 200ul methanol to fix it. After fixing for 3min, pour away the excess liquid and dry it.
4. Dye with Alcian Blue Staining Solution for 10-20min, and wash slightly with distilled water for 5-10s.
5. Redyeing with Red Staining Solution for 5-10min and slightly washing with distilled water for 5-10s.
6. Direct drying for microscopic inspection or neutral gum seal microscopic inspection after drying.

## Result

Cryptococcus neoformans capsule	Blue
Nucleus,Thallus	Red
Acidic mucus and cartilage	Blue

## Note

1. It is recommended to take 1-2 samples for preliminary experiment.
2. For special cells, if the cell precipitation is insufficient, can appropriately increase the centrifugation force or prolong the centrifugation time.

