V02

抗酸染色试剂盒（Ziehl-Neelsen 热染法）

货号：G1274
规格：3×50mL
保存：室温，避光保存，有效期 1 年。

产品组成：

名称	3×50mL	保存
试剂(A): Ziehl－Neelsen复红染色液	50mL	室温，避光
试剂(B): Ziehl－Neelsen脱色液	50mL	室温
试剂(C): 亚甲蓝染色液	50mL	室温，避光

产品介绍：

分枝杆菌的细胞壁内含有大量脂质包围在肽聚糖的外面，所以分枝杆菌一般不易着色。分枝杆菌中的分枝菌酸与染料一旦结合后，就很难被酸性脱色液脱色，故名抗酸染色。传统的染色方法要经过加热和延长染色时间来促使其着色，其中最具代表性的是结核杆菌Ziehl－Neelsen染色法，该法是WHO和中国结核病防治规划中推荐的热染方法。

抗酸染色液(Ziehl－Neelsen热染法)属于热染色液，其染色原理是在加热条件下，分枝菌酸与复红结合成复合物，经亚甲蓝复染后，分枝杆菌仍然为红色，而其他细菌及背景中的物质为蓝色。该试剂盒更适用于冷染法效果不佳的情况。

操作步骤：（*仅供参考*）

1. 接种环挑取待检样本，涂布于载玻片上，涂片加热固定。
2. 滴加Ziehl－Neelsen复红染色液，用火焰微热至出现蒸汽，一般该染色过程至少5min (必要时应补加染液、以防止染液蒸干)。蒸馏水冲洗。
3. 用Ziehl－Neelsen脱色液脱色至无红色为止，一般1min即可。蒸馏水冲洗。
4. 用亚甲蓝染色液染色1min。蒸馏水冲洗。
5. 轻轻吸干水分，自然干燥。油镜镜检。

染色结果：

抗酸菌	红色
背景及非抗酸菌	蓝色

注意事项：

1. 每次使用后盖紧试剂瓶，以防试剂挥发和污染。
2. 如难以实现操作步骤2中的火焰加热，可提前分取少量复红染色液至EP管中，封口后80-100℃预热5-10min。孵育盒内加80-100℃热水进行保温染色(必要时应补加染液、以防止染液蒸干)。
3. 石蜡切片推荐使用松节油替代二甲苯用于脱蜡和透明，避免过度脱脂导致假阴性。
4. 上述试剂均对人体有刺激性，请注意适当防护。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

Acid-Fast Bacillus(AFB)Stain Kit(Ziehl-Neelsen's Method)

Cat:G1274
Size:3×50mL
Storage:RT, avoid light, valid for at least 1 year.

Kit Components

Reagent	3×50mL	Storage
Reagent A: Ziehl-Neelsen Fuchsin Solution	50mL	RT , avoid light
Reagent B: Ziehl-Neelsen Destaining Solution	50mL	RT
Reagent C: Methylene Blue Solution	50mL	RT , avoid light

Introduction

The cell walls of mycobacteria contain a large amount of lipid in the outside of the peptidoglycan, so it is not easy staining. When the mycobacterium acid of mycobacteria combines with dye,it is difficult to be destained by acidic destaining solution. This method is called Acid-Fast Bacilius Stain. Traditional dyeing methods need to promote staining by heating and extending the time. The most representative method is Ziehl-Neelsen method, which is recommended in the WHO and China Tuberculosis Control Program.

Acid-Fast Bacillus(AFB)Stain Kit(Ziehl-Neelsen's Method) is a kind of thermal dyeing solution. The dyeing principle is that under heating condition, the mycobacterium acid combines with Fuchsin Stain and binds to a complex. Mycobacterium remains red after Methylene Blue re-dyeing, and the others are blue. The kit is more suitable when the effect of cold dyeing is not good.

Protocol(*for reference only*)

1. Pick up the samples to be examined with the inoculation ring and coat them on the glass slide. Then heat and fix the section.
2. Add Ziehl-Neelsen Fuchsin Solution to the section and heat it slightly with flame until vapor appears. Generally, the dyeing process should be at least 5 min (if necessary, add Ziehl-Neelsen Fuchsin Solution to prevent the evaporation of dyeing solution).Rinse with distilled water.
3. Destain with Ziehl-Neelsen Destaining Solution until there is no red, usually for 1 min. Rinse with distilled water.
4. Dyeing with Methylene Blue Solution for 1 min.Rinse with distilled water.
5. Gently absorb water and naturally dry. View under the oil immersion microscope.

Result

Acid-fast bacteria	Red
Background	Blue

Note

1. Cover the reagent bottle tightly after each use to prevent reagent evaporation and contamination.
2. If it is difficult to achieve flame heating in operation step 2. A small amount of fuchsin dye solution can be separated in advance into the EP tube, and preheated at 80-100 ℃ for 5-10min after sealing. Add 80-100 ℃ hot water to the incubation box for thermal insulation dyeing (add dye solution if necessary to prevent the dye solution from drying).
3. It is recommended to use turpentine instead of xylene for dewaxing and transparency in paraffin sections to avoid false negatives caused by excessive defatting.
4. The above reagents are irritating to human body. Please pay attention to appropriate protection.
5. For your safety and health, please wear experimental clothes and disposable gloves.