V02

# 中性粒细胞碱性磷酸酶染色试剂盒

**货号：**G2430
**规格：**4×10mL
**保存：** -20℃，避光保存，有效期 6 个月。

## 产品组成：

| 名称 | | 4×10mL | 保存 |
|---|---|---|---|
| 试剂(A): NAP 固定液 | | 10mL | 室温，避光 |
| 试剂(B): NAP 孵育液 | B1: AS-BI 染色液 | 5mL | -20℃，避光 |
| | B2: FBB 染色液 | 5mL | -20℃，避光 |
| 临用前，按 B1:B2=1:1 比例混合，即为 NAP 孵育液，即配即用。 | | | |
| 试剂(C): 核固红染色液 | | 10mL | 室温，避光 |
| 试剂(D): 甲基绿染色液 | | 10mL | 室温，避光 |

## 产品介绍：

碱性磷酸酶（Alkaline phosphatase，简称 ALP 或 AKP）为一类磷酸酯酶，广泛分布于哺乳动物组织内，其活性所需最适 PH 9.2-9.8。此酶主要存在于物质交换活跃之处（细胞膜），如肠上皮和肾近曲小管的刷状缘、附睾上皮之静纤毛、肝的毛细胆管膜以及微动脉和毛细血管动脉部之内皮，还见于内质网、高尔基复合体、吞饮小泡、肠上皮之溶酶体、中性粒细胞之中性颗粒以及平滑肌之细胞膜。

中性粒细胞碱性磷酸酶染色试剂盒（NAP）不是采用金属沉淀法来显示碱性磷酸酶活性，而是采用偶氮偶联法（又称同时偶联法），其原理是在 PH 9.2-9.8 的碱性条件下，细胞内碱性磷酸酶可使 AB-BI 磷酸盐水解，释放出磷酸和萘酚，后者与偶联重氮盐生成有色产物，定位于细胞质中。该染液专门用于血液或骨髓细胞涂片中中性粒细胞碱性磷酸酶染色，结果较金属盐沉淀法可靠。

## 自备材料：

载玻片、显微镜

## 操作步骤：（*仅供参考*）

*试剂(C)：核固红染色液可能会由于絮凝产生悬浮物或少量沉淀，建议取上清使用或沸水浴 5-10min 后晾至 30-40℃使用。（见注意事项 2）*

1. 制备新鲜血液或骨髓细胞涂片，NAP 固定液固定 30s，充分水洗。
2. 滴加配制好的 NAP 孵育液，避光孵育 15-20min，水洗。
3. 核固红染色液复染 5-8min 或甲基绿染色液复染 3-5min。
4. 水洗、晾干、镜检。

## 染色结果：

| | |
|---|---|
| NAP 阳性颗粒 | 蓝色 |
| 细胞核 | 红色(核固红)或绿色(甲基绿) |

一般以积分报告结果，根据 100 个中性粒细胞阳性颗粒进行 0-4 计分。

| 细胞分值 | 染色特点 |
|---|---|
| 0 | 无颗粒 |
| 1 | 稍有颗粒 |
| 2 | 中等程度颗粒 |
| 3 | 多数颗粒 |
| 4 | 充满颗粒 |

## 临床意义：

1. 类白血病反应积分明显增高，未经治疗的慢性粒细胞白血病积分明显减低。



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2. 试剂(C): 核固红染色液为胶体性质溶液，低温（低于 25℃）保存或长期储存由于絮凝产生悬浮物或少量沉淀，属于正常现象，一般不影响使用。如移液器吸取观察到明显浑浊，可拧紧瓶盖沸水浴 5-10min 重新制备分散均匀的胶体溶液来恢复使用。
3. 急性细菌性感染积分明显增高，病毒性感染积分多正常或减低。
4. 再生障碍性贫血积分常增高，PNH、MDS 积分常减低。

**注意事项：**

1. 血液或骨髓细胞涂片应新鲜，薄厚适宜，及时固定，否则会影响酶的活性。
2. 培养细胞染色操作过程中，清洗、染色等步骤都应轻微，以免损伤或丢失细胞。
3. NAP 孵育液易失效或降低阳性强度，即配即用，不宜久置。
4. 复染时，核固红染色液或甲基绿染色液二者取其一。
5. 每次染色时，应有阳性对照片。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作

V02

# Neutrophil Alkaline Phosphatase Stain Kit

**Cat:** G2430
**Size:**4×10mL
**Storage:** -20℃, avoid light, valid for 6 months.

## Kit Components

| Reagent | | 4×10mL | Storage |
|---|---|---|---|
| Reagent(A): NAP Fixative | | 10mL | RT, avoid light |
| Reagent(B): NAP Incubation Solution | B1: AS-BI Solution | 5mL | -20℃, avoid light |
| | B2: FBB Solution | 5mL | -20℃, avoid light |
| Before use, mix B1 with B2 in equal amount to form NAP Incubation Solution. It is ready to use. | | | |
| Reagent(C): Nuclear Fast Red Solution | | 10mL | RT, avoid light |
| Reagent(D): Methyl Green Solution | | 10mL | RT, avoid light |

## Introduction

Alkaline phosphatase (ALP or AKP) is a kind of phosphatase, which is widely distributed in mammalian tissues, and the optimal pH of its activity is 9.2-9.8. This enzyme is mainly found in the active sites of material exchange (cell membrane), such as the brush border of intestinal epithelium and renal proximal convoluted tubules, the static cilia of epididymal epithelium, the membrane of bile capillaries of liver, the endothelium of arterioles and capillaries, and also in the endoplasmic reticulum, golgi complex, swallowing vesicles, lysosomes of intestinal epithelium, neutrophils of neutrophils, and the membrane of smooth muscle.

This kit is not used to display alkaline phosphatase activity by metal precipitation method, but by azo coupling method (also known as simultaneous coupling method). Its principle is that under the alkaline condition of pH 9.2-9.8, alkaline phosphatase in cells can hydrolyze AB-BI phosphate to release phosphoric acid and naphthol, the latter can generate colored products with coupling diazonium salt, which is located in cytoplasm. The staining solution is specially used for alkaline phosphatase staining of neutrophils in blood or bone marrow cell smear, and the result is more reliable than that of metal salt precipitation method.

## Self Provided Materials

Slide, Microscope

## Protocol(*for reference only*)

*Reagent(C): Nuclear Fast Red Solution may produce suspended solids or a small amount of precipitation due to flocculation. It is recommended to take supernatant or boil water bath for 5-10min and then air it to 30-40 ℃. (see Note 2)*

1. Make fresh blood or bone marrow cell smear, fix with NAP Fixative for 30s and wash with water thoroughly.
2. Drop the prepared NAP Incubation Solution and incubate in dark for 15-20min, then wash with water.
3. Re-dyeing with Nuclear Fast Red Solution for 5-8 min or re-dyeing with Methyl Green Solution for 3-5 min.
4. Wash, dry and view under the microscope.

## Result

| | |
|---|---|
| NAP Positive particles | Blue |
| Nucleus | Red or Green |

Generally record the result by score and score with 0-4 according to 100 neutrophil positive particles.

| Score level | Dyeing characteristics |
|---|---|
| 0 | No particle |
| 1 | A little few particles |
| 2 | Medium-degree particles |
| 3 | Most particles |
| 4 | Full of particles |

### Clinical significance

1. The score of leukemoid reaction significantly increases and untreated chronic myeloid leukemia significantly decreases.
2. The score of acute bacterial infection significantly increases and viral infection mostly keep normal or decrease .



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3. The score of aplastic anemia always increases and the score of PNH and MDS always decrease.

## Note

1. The smear of blood or bone marrow cells should be fresh and in appropriate thickness. Fix in time, otherwise it will affect the activity of enzyme.
2. Reagent(C): Nuclear Fast Red Solution is a colloidal solution, which is stored at low temperature (lower than 25 ℃) or stored for a long time. Suspended solids or a small amount of precipitation are generated due to flocculation, which is a normal phenomenon and generally does not affect the use. If the colloid solution is evenly dispersed in the boiling bath, tighten the bottle cap for 5-10min to recover the turbid solution.
3. In the process of cultured cell staining, the steps of cleaning and staining should be slight to avoid cell damage or loss.
4. NAP Incubation Solution is easy to lose effect or reduce the positive intensity. It is ready to use, not stored for long time.
5. Choose one of Nuclear Fast Red Solution and Methyl Green Solution when redyeing.
6. There should be a positive control section for each staining.
7. For your safety and health, please wear experimental clothes and disposable gloves.