酸性α-乙酸萘酚酯酶染色试剂盒(ANAE 法)

货号： G2390
规格： 3×10mL
保存： 2-8℃, 避光保存, 有效期 6 个月。

产品组成：

名称		3×10mL	保存
试剂(A): ANAE 固定液		10mL	室温，避光
试剂(B): ANAE 孵化液	B1：新品红溶液	0.05mL	2-8℃，避光
	B2：Nitrite 溶液	0.05mL	2-8℃，避光
	B3：α-NAE 溶液	0.5mL	2-8℃，避光
	B4：ANAE 缓冲液	9.5mL	2-8℃
临用前，按 B1：B2：B3：B4=1：1：10：190 充分混合，即为 ANAE 孵化液，即配即用。注意应先 B1：B2=1:1 混匀后，再与 B3、B4 混匀。			
试剂(C):甲基绿染色液		10mL	室温，避光
试剂(D): ANAE 抑制剂		0.2mL	室温，避光

产品介绍：

酯酶主要分为非特异性酯酶(non-specific esterase)、酯酶(lipase)、胆碱酯酶(choli-esterase)。酸性α-乙酸萘酚酯酶染色液(ANAE 法)又称非特异性酯酶染色液，其原理是酸性条件下细胞中的酸性酯酶将α-乙酸萘酚水解产生α-萘酚，α-萘酚再与六偶氮副品红偶联，生成不溶性红色沉淀，定位于细胞质。本染色试剂盒对酯酶染色无特异性，可用于血液、骨髓或细胞涂片、冰冻切片的非特异性酯酶染色，亦可用于氟化钠抑制试验。该染色液仅用于科研领域，不适用于临床诊断或其他用途。

自备材料：

载玻片、湿盒、显微镜

操作步骤：（*仅供参考*）

1. 血液、骨髓或细胞涂片、冰冻切片入 ANAE 固定液固定 10-15min。
2. 水洗 5min。
3. 入配置好的 ANAE 孵育液，放入湿盒中，室温避光孵育 1h，水洗。
4. 入甲基绿染色液复染 5-15min，水洗，镜检。

染色结果：

细胞质	暗红色/棕色
细胞核	绿色

氟化钠抑制实验：

按 ANAE 抑制剂：ANAE 孵育液=1：25 的比例，在 ANAE 孵育液中加入 ANAE 抑制剂，其余按上述染色法进行。

注意事项：

1. 血液或骨髓细胞涂片应新鲜，薄厚适宜，一般 2 天内染色，否则会影响酶的活性。
2. ANAE 孵育液易失效或降低阳性程度，即配即用，不宜久置。
3. ANAE 孵育液配置后易出现浑浊，但不会影响染色效果。
4. 单核细胞为中度阳性至强阳性，对 ANAE 抑制剂敏感。正常粒细胞呈阴性反应。
5. 每次染色时，应有阳性对照。

Acid α-Naphthol Acetate Esterase Stain Kit(ANAE Method)

Cat: G2390
Size: 3×10mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		3×10mL	Storage
Reagent A:ANAE Fixative		10mL	RT , avoid light
Reagent B:ANAE Incubation Solution	B1:Pararosaniline Solution	0.05mL	2-8℃, avoid light
	B2:Nitrite Solution	0.05mL	2-8℃, avoid light
	B3:α-NAE Solution	0.5mL	2-8℃, avoid light
	B4:ANAE Buffer	9.5mL	2-8℃
Before use, mix B1, B2, B3 and B4 as the radio of 1:1:10:190 fully to form ANAE Incubation Solution. It is ready to use. *Note: mix with B3 and B4 after mixing B1 and B2 in equal amount*			
Reagent C:Methyl Green Staining Solution		10mL	RT , avoid light
Reagent D:ANAE Inhibitor		0.2mL	RT , avoid light

Self Provided Materials

Slide, Wet Box, Microscope.

Introduction

Esterase is mainly divided into non-specific esterase, lipase and choli-esterase. Acid α-naphthol Acetate Esterase Stain Kit(ANAE Method) is also called non-specific esterase staining solution. Its principle is that under acid conditions, acid esterase in cells hydrolyzes α - naphthol acetate to produce α - naphthol, then α - naphthol is coupled with azo fuchsin to form insoluble red precipitate, which is located in cytoplasm. This staining solution has no specificity for esterase staining. It can be used for non-specific esterase staining of blood, bone marrow or cell smear, frozen section, and sodium fluoride inhibition test. The staining solution is only used in scientific research, not for clinical diagnosis or other purposes.

Protocol(*for reference only*)

1. Fix blood, bone marrow or cell smear frozen section with ANAE Fixative for 10-15 min.
2. Wash with water for 5min.
3. Add the prepared ANAE Incubation Solution and put into a wet box, incubate at room temperature avoiding light for 1h. Then wash with water.
4. Re-dyeing with Methyl Green Staining Solution for 5-15min, wash with water and view under the microscope.

Result

Cytoplasm	Dark Red or Brown
Nucleus	Green

Sodium fluoride inhibition test:

According to the ratio of ANAE Inhibitor: ANAE Incubation Solution = 1:25, add the ANAE Inhibitor into ANAE Incubation Solution, and the rest follow the above steps.

Note

1. The smear of blood or bone marrow cells should be fresh and in appropriate thickness, and generally stain within 2 days, otherwise the enzyme activity will be affected.
2. ANAE Incubation Solution is easy to lose effect or reduce the positive degree. It is ready to use, not stored for long time.
3. ANAE Incubation Solution is easy to appear turbid after configuration, but it will not affect the dyeing effect.
4. Monocytes are moderately positive to strongly positive and sensitive to ANAE inhibitors. The normal granulocytes show negative reaction.
5. There should be positive control for each staining.