精子形态学染色试剂盒(巴氏法)

货号： G2571
规格： 4×20mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称	4×20mL	保存
试剂(A): 苏木素染色液	20mL	室温，避光
试剂(B): 蓝化液	20mL	室温
试剂(C): 橘黄G6染色液	20mL	室温，避光
试剂(D): 改良EA50染色液	20mL	室温，避光

产品介绍：

细胞学常规染色普遍使用巴氏(Papanicolaou)法，Papanicolaou Stain 最初仅用于检测阴道上皮雌激素水平以及生殖道念珠菌、滴虫等病原体。橘黄 G6 与 EA36 或 EA50 联合使用，可将胞浆染成颜色鲜明的绿色、蓝色和粉色。目前大多数实验室采用成品染液，所以每种染液应注意其改良后的最佳条件。最终胞浆染色应透明可见，核染色质应很容易辨别出来。目前改良的巴氏染色液含有多种离子，具有多色性染色效能。染色后胞质鲜艳、透明性好，核膜、核仁、染色质结构清晰。

精子形态学染色试剂盒(巴氏法) 因精子及细胞内不同等电点的蛋白质在相同的酸度下带不同的电荷，能选择性地结合相应的染料而着色。胞核由酸性物质组成，它与碱性染料的亲和力较强；而胞浆则相反，它含有碱性物质和酸性染料的亲和力较大，细胞质染色特别采用针对于精子染色的改良 EA50 染色液，细胞核染色采用自主研发的无毒改良型苏木素染色液，特别适用于精子的染色，亦可用于胸水、腹水、痰液等细胞样本的染色。

自备材料：

固定液(如 95%乙醇)、系列乙醇、酸性乙醇分化液

操作步骤：(*仅供参考*)

1. 新鲜精液标本完全液化或贮存于-20℃的精液标本复温（室温）。
2. 需要对精子进行洗涤后制片，方法如下：(1) 在试管内加入精液 0.5mL 和生理盐水 10 mL，充分混匀。(2) 2000 转离心 10min，去掉大部分的上清液（必要时重复①②步骤）。 (3) 轻轻弹动试管使离心后的精子团悬浮于剩余的盐水中，若精子浓度过高，则以生理盐水适当调整精子密度。
3. 在一张洁净的载玻片上，加入 10-20μl 精液，并将其均匀涂成一个圆形样本区，涂制好的玻片水平放置，空气中自然干燥。
4. 将干燥后的涂片在 95%乙醇溶液中固定 5-10 分钟。
5. 系列乙醇（80%、70%、50%）从高到低复水，每梯度 1min。
6. 蒸馏水或自来水浸泡或冲洗 1min。
7. 苏木素染色液染色 3-5min。
8. 蒸馏水冲洗 30s。
9. 酸性乙醇分化液分化约 4-5s，建议镜下观察分化至适当程度。
10. 蓝化液中蓝化 4min。
11. 自来水冲洗 2min。
12. 50%、70%、80%、95%系列乙醇脱水，每梯度 1min。
13. 橘黄 G6 染色液染色 2min。
14. 95%的乙醇(I)、(II)冲洗各 2min。
15. 改良 EA50 染色液染色 5min。
16. 95%的乙醇(Ⅰ)、(Ⅱ)脱水各 1min。
17. 无水乙醇(Ⅰ)、(Ⅱ)脱水各 1min。
18. 二甲苯透明，中性树脂封片。

染色结果：

精子顶体区	淡蓝色
头部非顶体区 （顶体后区）	深蓝色
中段	略呈红色
精子体尾部	蓝色或淡红色
通常位于头部下部或围绕中段的过量残留胞浆染成红色。	

注意事项：

1. 所有染液均需过滤，需经常更换染液。
2. 酸性乙醇分化液通常为盐酸乙醇，可购置 G1860-0.5%酸性乙醇分化液或自行配置使用。
3. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Sperm Morphology Stain Kit(Papanicolaou Method)

Cat:G2571
Size:4×20mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent	4×20mL	Storage
Reagent(A): Hematoxylin Staining Solution	20mL	RT, avoid light
Reagent(B): Bluing Solution	20mL	RT
Reagent(C): Orange G6 Solution	20mL	RT, avoid light
Reagent(D): Modified EA50 Solution	20mL	RT, avoid light

Self Provided Materials

Fixative(such as 95% ethanol), Series of Ethanol,Acid Ethanol Fifferentiation Solution

Introduction

Papanicolaou method is widely used in routine cytological staining. Papanicolaou stain was initially only used to detect estrogen level in vaginal epithelium and pathogens such as Candida and trichomonad in genital tract. The combination of orange G6 and EA36 or EA50 can dye the cytoplasm into bright green, blue and pink. At present, most laboratories use finished dye solution, so we should pay attention to the best conditions of each dye solution after improvement. The final cytoplasmic staining should be transparent, and the nuclear chromatin should be easily distinguished. At present, the modified papanicolaou solution contains many kinds of ions, which has polychromatic dyeing efficiency. After staining, the cytoplasm is bright and transparent, and the structure of nuclear membrane, nucleolus and chromatin is clear.

Sperm Morphology Stain Kit(Papanicolaou Method) can selectively combine with the corresponding dyes to dye sperm and proteins with different isoelectric points in the same acidity. The nucleus is composed of acidic substances, which have a strong affinity with alkaline dyes; on the contrary, the cytoplasm has a large affinity with alkaline substances and acid dyes. The cytoplasm is dyed with the modified EA50 staining solution specially for sperm staining, and the nucleus is dyed with the non-toxic modified hematoxylin staining solution independently developed, which is especially suitable for sperm staining, as well as the cell samples of pleural effusion and ascites, sputum.

Protocol(*for reference only*)

1. Completely liquefy the fresh semen sample or restore the semen sample stored at - 20 ℃ to room temperature.
2. It is necessary to wash the sperm before making a section. The methods are as follows: (1) add 0.5mL semen and 10mL physiological saline into the test tube and mix them well. (2) centrifugation for 10 min at 2000 revolutions, and remove most of the supernatant (repeat step ① and step ② if necessary) (3) gently flick the test tube to suspend the centrifuged sperm mass in the remaining physiological saline. If the sperm concentration is too high, adjust the sperm density with physiological saline.
3. Add 10-20 μl semen onto a clean slide and evenly smear it into a circular sample area. Place the slide horizontally and dry it naturally in the air.
4. Fix the dried smear in 95% ethanol for 5-10 min.
5. Soak in series of ethanol(80%,70%,50%) from high to low for 1 min each.
6. Soak or wash in distilled water or tap water for 1min.
7. Stain with Hematoxylin Staining Solution for 3-5min.
8. Wash with distilled water for 30s.
9. Differentiate by hydrochloric acid ethanol differentiation solution for about 4-5s. It is suggested to view the differentiation to an appropriate degree under the microscope.
10. Blue in Bluing Solution for 4min.
11. Wash with tap water for 2min.
12. Dehydrate in series of ethanol(50%,70%,80%,95%) for 1min each.
13. Stain with Orange G6 Solution for 2min.
14. Rinse with 95% ethanol (I) and (II) for 2min respectively.

15. Stain with Modified EA50 Solution for 5min.
16. Dehydrate in 95% ethanol (I) and (II) for 1 min respectively.
17. Dehydrate in absolute ethanol (I) and (II) for 1 min respectively.
18. Transparent by xylene and seal with resinene.

Result

Acrosome region of sperm head	Light Blue
Postacrosomal region	Deep Blue
Middle part	Slight Red
Rear part	Blue or Slight Red
The excessive residual cytoplasm that usually located in the lower part of the head or around the middle part is dyed orange red	

Note

1. All dye solutions should be filtered and changed frequently.
2. Acid ethanol differentiation solution is usually hydrochloric acid ethanol, which can be purchased from G1860-0.5% acid ethanol differentiation solution or self-made.
3. For your safety and health, please wear experimental clothes and disposable gloves.