精子形态学快速染色液(Diff-Quik法)

货号： G2572
规格： 3×20mL/3×100mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称	3×20mL	3×100mL	保存
试剂(A)：迪夫固定液	20mL	100mL	室温，避光
试剂(B)：迪夫染色液 I	20mL	100mL	室温，避光
试剂(C)：迪夫染色液 II	20mL	100mL	室温，避光

产品介绍：

Diff-Quik 染色是在 Wright 染色基础上改良而来的一种快速染色方法，是细胞学检查中常用的染色方法之一，染液是采用世界卫生组织(WHO)推荐的快速染色方法而配制，与 Wright Stain 类似都是利用 Romanowsky Stain 技术原理改良而来的，染色结果与瑞氏染色液也极其相似，但迪夫快速染色所需的时间极短，一般 90s 以内即可完成染色。 精子形态的评估可以采用 Diff-Quik 染色液。

精子形态学快速染色液(Diff-Quik 法)属于 WHO 推荐的精子形态学染色，其染色原理是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；嗜酸性蛋白质解离的氨基带正电荷，能与带负电荷的酸性染料(如嗜酸性氧杂蒽、伊红)结合而被染成红色；嗜碱性蛋白质解离的羧基带负电荷，能与带正电荷的碱性染料(嗜碱性硫氮杂苯、亚甲蓝)结合而被染成蓝色；嗜中性蛋白质解离的带正电荷的氨基和带负电荷的羧基相等，同时结合相等的酸性染料和碱性染料而呈紫红色，但因解离电荷相等，故着色较弱。该染色液主要用于精子(细胞)形态的评估，含固定液，非常适合用于批量浸染，且背景清晰无沉渣，该染色液仅用于科研领域，不宜用于临床诊断或其他用途。

自备材料：

载玻片、蒸馏水、显微镜

操作步骤：(*仅供参考*)

1、 制备涂片：彻底清洗2张载玻片，再用70%的乙醇洗涤，晾干。滴加5-20μL于玻片上，用第2张载玻片的边缘在清洁载玻片表面拖拉一滴精液，制成涂片。如果精子密度过高，可用生理盐水适当稀释。
2、 入迪夫固定液或自然干燥，固定15-20s。
3、 将玻片直立于吸水纸上以去除多余的液体。
4、 玻片入迪夫染色液 I 染色10-20s，将玻片直立于吸水纸上以去除多余的液体。
5、 玻片入迪夫染色液 II 染色5-10s，将玻片直立于吸水纸上以去除多余的液体。
6、 流水浸洗10-15次以去除多余的染液。
7、 将玻片直立于吸水纸上以去除多余的水分，并使其完全干燥，显微镜下观察。

染色结果：

精子头部顶体区	淡蓝色
精子顶体区后区	深蓝色
精子中段	可能为淡红色
精子尾部	蓝色或淡红色不等

注意事项：

1、 如果精子密度>20×10^6/mL，应取5μL；如果精子密度<20×10^6/mL，应取5-20μL。
2、 迪夫固定液是精子Diff-Quik法的专用固定液，如需大量可用甲醇替代。
3、 精子样本应新鲜，精子涂片应厚薄均匀，以免影响染色效果，如果精子密度过高，可采用生理盐水适当稀释。

4、 涂片染色中请勿先去除染液或直接对涂片用力冲洗。不能先倒掉染液，以免染料沉着于涂片上。
5、 染色液可重复使用，但不能多次重复，若有沉淀物应过滤后使用。
6、 染色过深可用甲醇或酒精适当脱色，最好不复染。
7、 如果染色过深或过浅，应调整染色时间或工作液浓度。
8、 pH 值对染色有一定影响，载玻片应清洁、无酸碱污染，以免影响染色效果。
9、 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 (Diff-Quick Method)

Cat: G2572
Size:3×20mL/3×100mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent	3×20mL	3×100mL	Storage
Reagent(A): Diff-Quick Fixative	20mL	100mL	RT
Reagent(B): Diff-Quick Ⅰ	20mL	100mL	RT, avoid light
Reagent(C): Diff-Quick Ⅱ	20mL	100mL	RT, avoid light

Introduction

Diff-Quick Staining is a fast staining method improved base on Wright Staining. It is one of the common staining methods in cytology test. The dye solution is made by the fast staining method recommended by WHO. Similar to Wright Stain, it is improved base on Romanowsky Stain technology principle. The staining result is very similar to that of Wright Stain, but Diff-Quick Stain takes a very short time, usually within 90 seconds. Diff Quick Stain can be used to evaluate sperm morphology.

Sperm Morphology Stain (Diff-Quick Method) is a sperm morphology staining solution recommended by WHO. Its dyeing principle is that sperm and proteins with different isoelectric points in cells have different charges under the same acidity. They can selectively combine with corresponding dyes to dye; the amino group dissociated by eosinophilic protein has positive charge, which can mix with acid dyes containing negative charge (such as eosinophilic xanthene and eosin) and be dyed red ; the carboxyl group dissociated by basophil protein has negative charge, which can combine with basic dyes containing positive charge (basophil thioazabenzene and methylene blue) and be dyed blue; the number of amino group with positive charge and carboxyl group with negative charge dissociated by the neutral protein are the same. They simultaneously combine with acid dye and basic dye to be dyed purplish red, but the color is weak due to the equal dissociation charge. The dye is mainly used for sperm (cell) morphology evaluation, including fixative, which is very suitable for batch dyeing, and the background is clear without sediment. The dye is only used in scientific research, not for clinical diagnosis or other purposes.

Self Provided Materials

Slide, Distilled water, Microscope.

Protocol(*for reference only*)

1. Prepare smear: wash 2 slides thoroughly, then wash with 70% ethanol and dry. Add 5-20 μL onto the slide, drag a drop of semen on the surface of the clean slide with the edge of the second slide, and make a smear. If the sperm density is too high, can dilute with physiological saline.
2. Put the smear in Diff-Quick Fixative or dry naturally, and fix for 15-20s.
3. Place the smear vertically on the absorbent paper to remove the excess liquid.
4. Stain with Diff-Quick Ⅰ for 10-20s and place the smear vertically on the absorbent paper to remove the excess liquid.
5. Stain with Diff-Quick Ⅱ for 5-10s and place the smear vertically on the absorbent paper to remove the excess liquid.
6. Wash with running water for 10-15 times to remove excess dye solution.
7. Place the smear vertically on the absorbent paper to remove the excess liquid and make it completely dry. View under the microscope.

Result

Acrosome region of sperm head	Light Blue
Postacrosomal region	Deep Blue
Middle part	Maybe Slight Red
Rear part	Blue or Slight Red

Note

1. If the sperm density is more than 20×10^6 / mL, take 5 μL; if the sperm density is less than 20×10^6 / mL, take

5-20 μ L.

2. Diff-Quick Fixative is a special fixative for Sperm Diff-Quick Staining Method. If use a lot ,can replace with methanol.
3. The sperm sample should be fresh, and the sperm smear should be even and thick, so as not to affect the staining effect. If the sperm density is too high, adjust the sperm density with physiological saline.
4. Avoid removing the dye solution directly or wash the smear vigorously. Do not discard the dye first, so as to prevent the dye from settling on the smear.
5. The dye solution can be reused, but it can not be repeated many times. If there is sediment, filter before use.
6. If the dyed color is too deep, it can be decolorized properly with methanol or alcohol, and it is better not to be re-dyed.
7. If the dyed color is too deep or too shallow, adjust the dyeing time or working solution concentration.
8. The pH value has certain influence on the dyeing. The slide should be clean and free of acid and alkali pollution to avoid affecting the dyeing effect.
9. For your safety and health, please wear experimental clothes and disposable gloves.