细胞迁移与侵袭实验染色试剂盒（适用于 Transwell 实验）

货号： G4740
规格： 3×20mL/3×50mL
保存： 室温，避光保存，有效期至少 1 年。

产品组成：

名称		3×20mL	3×50ml	保存
试剂(A):细胞固定液		20mL	50mL	2-8℃
试剂(B):染色工作液	试剂(B1):原液（5×）	4mL	10mL	2-8℃，避光
	试剂(B2):稀释液	16mL	40mL	室温
临用时将试剂 B1、B2 按照 1：4 的比例配成染色工作液，建议 6 小时内使用完毕。				
试剂(C):洗脱液		20mL	50mL	室温

产品介绍：

Transwell 是一种能在体外模拟机体许多黏膜及生物屏障系统的实验技术，这项技术的主要材料是 Transwell 小室，其外形为一个可放置在孔板里的小杯子，杯子底层为一张有通透性的膜，这层膜带有微孔。可以进行共培养、细胞趋化、细胞迁移、细胞侵袭等多种方面的研究。

细胞迁移与侵袭实验染色试剂盒（适用于 Transwell 实验）为 transwell 相关实验配套染色试剂盒，主要染色成分为结晶紫，可以便捷快速的对迁移或侵袭实验的结果进行可视化展示。同时配有洗脱液可以对迁移率和趋化系数进行半定量计算。

操作步骤：（*仅供参考*）

1. 将 Transwell 小室从板中取出，用 1×PBS 浸洗 3 次，每次 1 分钟，用棉签轻轻擦去上层未迁移的细胞。
2. 根据孔径大小加入适量的 4℃预冷的固定液固定 20 分钟，1×PBS 稍洗。（见注意事项 1）
3. 按照 B1:B2=1:4 的比例配置与所用固定液等量的染色工作液，室温浸染 5-15 分钟，蒸馏水洗 2 次，每次 1 分钟。（见注意事项 2）
4. 浸于蒸馏水或 1×PBS 中镜下观察，拍照计算迁移比率。
5. （可选）如需封片可蒸馏水洗后晾干小室，将膜用剪刀小心的沿内侧边缘剪下，下表面（细胞面）朝上贴附在载玻片上，中性树脂封片。
6. （可选）也可以将小室浸于与所用固定液等量的洗脱液中，充分洗脱，收集洗脱后的液体用酶标仪测 570nm 处吸收峰可进行细胞迁移率半定量计算。（见注意事项 3）

染色结果：

细胞核	紫色
细胞质	淡紫色
背景	不着色

注意事项：

1. 不同孔径的 Transwell 小室染色所需液体量是不一样的，推荐参考值为六孔板每孔 1ml，24 孔板每孔 300ul，以此类推。推荐在处理和染色过程中保证上室下室液面尽量一致，通常建议六孔板上室加入 200-300ul，下室加入 700-800ul。
2. 环境温度和试剂温度均会对充分染色所需时间产生影响，但较短的染色时间只对着色的深浅造成影响，如观察颜色较浅可适当加温或延长染色时间复染来达到所需的效果，在 37℃浸染 15min 可保证充分着色。
3. 通常在 22-28℃的室温环境需要 10 分钟完成洗脱，温度过低或过高可适当延长或缩短。长时间浸泡对结果无影响。脱色不影响细胞状态，可重新染色封片观察。
4. 过长的培养时间可能会导致部分迁移或侵袭的细胞从膜底面脱落，可以通过适当缩短培养时间或在小

室底面包被一层纤维黏连蛋白增强粘附。

5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Cell Migration And Invasion Stain Kit (For Transwell)

Cat: G4740
Size: 3×20mL/3×50mL
Storage: RT, avoid light, valid for 1 year.

Kit components

Reagent		3×20mL	3×50ml	Storage
Reagent(A):Cell Fixative		20mL	50mL	2-8℃
Reagent(B): Stain Working Solution	B1:Stock Solution（5×）	4mL	10mL	2-8℃,avoid light
	B2: Diluent	16mL	40mL	RT
Before use, mix B1 with B2 as the radio of 1:4, which is Stain Working Solution, it is recommended to use within 6 hour.				
Reagent(C):Eluent		20mL	50mL	RT

Introduction

Transwell is an experimental technology that can simulate many mucosal and biological barrier systems in vitro. The main material of this technology is Transwell chamber, which is a small cup that can be placed in an orifice plate. The bottom layer of the cup is a permeable membrane with micro pores. It can be used to study co culture, cell chemotaxis, cell migration, cell invasion and so on.

TheCell Migration And Invasion Stain Kit (For Transwell) is a matching staining kit for Transwell related experiments. The main staining component is crystal violet, which can easily and quickly visualize the results of migration or invasion experiments. At the same time, with eluent, the mobility and chemotaxis coefficient can be calculated semi quantitatively.

Protocols*(for reference only)*

1. Take the Transwell chamber out of the plate and soak in 1×PBS for 3 times for 1 minute each time, then gently wipe off the non migrated cells in the upper layer with a cotton swab.
2. Add an appropriate amount of 4 ℃ precooled fixative according to the pore size and fix for 20 minutes, wash with 1 × PBS slightly. (*see note 1*)
3. According to the ratio of B1:B2 = 1:4, prepare the same amount of Stain Working Solution as the fixative used, soak dyeing at room temperature for 5-15 minutes, and wash twice with distilled water for 1 minute each time. (*see note 2*)
4. Immerse in distilled water or 1×PBS and observe under the microscope and photograph to calculate the migration ratio.
5. (*optional*) if it is required to seal, wash it with distilled water and dry the chamber. Carefully cut off the membrane along the inner edge with scissors, and stick the lower surface (cell surface) upward on the slide. Seal the slide with resinene.
6. (*optional*) the cell can also be immersed in the same amount of Eluent as the fixed solution used for full elution. The eluted liquid can be collected to measure the absorption peak at 570nm with an enzyme plotter for semi quantitative calculation of cell mobility. (*see note 3*)

Result

Nuclear	Purple
Cytoplasm	Light Purple
Background	Non-colored

Note

1. The amount of liquid required for Transwell chamber depends on different pore sizes. The recommended reference values are 1ml per hole for six hole plate, 300ul per hole for 24 hole plate, and so on. It is recommended to ensure that the liquid level in the upper chamber and the lower chamber is as consistent as possible during treatment and dyeing. Generally, it is recommended to add 200-300ul in the upper chamber and 700-800ul in the lower chamber of the six hole plate.
2. Both ambient temperature and reagent temperature will affect the time required for full dyeing, but a short

dyeing time only affects the color depth. If the color is observed to be light, it can be heated appropriately or the dyeing time can be extended to achieve the required effect. Soaking at 37 ℃ for 15min can ensure full dyeing.

3. It usually takes 10 minutes to complete the elution at room temperature of 22-28 ℃. If the temperature is too low or too high, it can be extended or shortened appropriately. Prolonged immersion had no effect on the results. Decolorization does not affect the cell state, which can be observed by re staining and sealing.
4. Too long culture time may cause some migrating or invasive cells to fall off from the bottom of the membrane. The adhesion can be enhanced by appropriately shortening the culture time or by a layer of fibronectin on the bottom of the chamber.
5. For your safety and health, please wear experimental clothes and disposable gloves.