V02

Cy5 酪胺信号放大试剂盒，HRP-羊抗兔 IgG

货号：G4867
规格：100T/300T
保存：-20℃，避光保存，有效期 1 年。开盖后有效期 3 个月。

产品组成：

名称		100T	300T	保存
试剂(A)：酪胺标记工作液	试剂(A1)：TYR-Cy5 储备液 500×	20uL	60uL	-20℃，避光
	试剂(A2)：染色增强液 100×	0.1mL	0.3mL	2-8℃，避光
	试剂(A3)：染色稀释液	10mL	30mL	2-8℃
临用前按照 A1:A2:A3=1:5:500 的比例混匀配制酪胺标记工作液，现配现用。				
试剂(B)：过氧化物酶增强封闭液		10mL	30ml	室温
试剂(C)：HRP-羊抗兔 IgG 100×		0.1mL	0.3mL	-20℃，避光

产品介绍：

酪酰胺信号放大（TSA）系统可用于检测荧光免疫细胞化学（ICC）、免疫组织化学（IHC）和原位杂交（ISH）技术中的低丰度靶点，可将信号灵敏度提高 100 倍。TSA 荧光试剂盒使用辣根过氧化物酶（HRP）直接催化固定化酶周围的荧光基团共价沉积，该标记过程迅速（小于 10min），沉积标记可直接在标准或共聚焦显微镜下观察。TSA 试剂盒可与传统染色方法结合用于多色成像，也可以顺序进行多个酪胺反应以标记一个样品上的不同靶标。

索莱宝酪酰胺信号放大（TSA）系列产品包括八款试剂盒：试剂盒 G4860/G4861 荧光标记为 fluorescein，在 494nm/517nm 下检测；试剂盒 G4862/G4863 荧光标记为 Trama，在 552nm/578nm 下检测；试剂盒 G4864/G4865 荧光标记为 Cyanine3，在 550nm/570nm 下检测；试剂盒 G4866/G4867 荧光标记为 Cyanine5，在 648nm/667nm 下检测。

自备试剂：

P1020-1×PBS、P1110-4%组织细胞固定液、P1080-通透剂（Triton X-100，强）、1×PBST、柠檬酸钠抗原修复液（1×）或 EDTA 抗原修复液、5%BSA 封闭液或无血清封闭液。上述试剂可自配也可采购市售成品试剂。

操作步骤：（*仅供参考*）

样本制备

1. (可选)准备一份阴性对照样本（不孵育一抗的样本）。（见注意事项 7）
2. 细胞样本制备：
   ① 1×PBS 润洗细胞 2 次，每次 2min。
   ② 吸去 PBS 后加入适量预冷的 4%组织细胞固定液，室温固定 15min。（见注意事项 1）
   ③ 1×PBS 润洗细胞 2 次，每次 2min。
3. 石蜡切片制备：
   ① 将石蜡切片放置在 60℃的烘箱中 30min。
   ② 室温下用二甲苯或环保组织透明脱蜡液浸泡脱蜡 2 次，每次 5min，以彻底脱蜡。
   ③ 室温下，系列乙醇复水：无水乙醇I、无水乙醇II、95%乙醇、90%乙醇、80%乙醇、70%乙醇、蒸馏水各 5min。1×PBS 浸洗 3min。
4. 冰冻切片制备：
   ① 从冰箱取出冰冻切片，放置 2-8℃冰箱平衡 10min。1×PBS 润洗切片 2 次，每次 2min。
   ② 吸去 PBS 后滴加适量预冷的 4%组织细胞固定液，室温固定 15min。（见注意事项 2）
   ③ 1×PBS 润洗样本 2 次，每次 2min。
5. 用免疫组化笔在切片背面样本周围描绘样品轮廓，以便后续进行定位和成像。

修复与封闭

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1. (可选)抗原修复：将适配一抗的抗原修复液加热至沸腾，将制备好的片子置于缓冲液中，间断煮沸 10min。抗原修复后连修复液一同放置室温中缓慢降温。（见注意事项 3）
2. (可选)细胞通透：样本滴加适量通透剂（Triton X-100，强）室温孵育 10min。（见注意事项 4）
3. (可选)过氧化物酶灭活：每样本滴加适量试剂（B）：过氧化物酶增强封闭液室温封闭 5-10min，1×PBS 润洗样本 2 次，每次 2min。
4. 位点封闭：使用封闭专用血清或 BSA 封闭液或无血清封闭液覆盖样本室温封闭 10min。

免疫标记

1. 用封闭液稀释一抗，滴加覆盖样品后在 37℃孵育 1h 或 2-8℃过夜（8-12h）。
2. 室温下 1×PBS 洗涤 3 次，每次 5min。
3. 用封闭液稀释试剂(C):HRP-羊抗兔 IgG 100×，滴加覆盖样品后在 37℃孵育 40min。
4. 室温下 1×PBS 洗涤 3 次，每次 5min。
5. 每个样品准备 100μL-300μL 酪胺标记工作液。工作液在室温下避光保存，最长可保存过夜。
6. 滴加孵育液覆盖样本在室温下避光孵育 10min。
7. 在室温下用 1×PBS 洗涤 3 次，每次 5min。
8. 显微镜成像。对于载玻片上的组织样本，请使用水性明胶封片剂或抗荧光衰减封片剂封片后再显微成像。

注意事项：

1. 操作步骤中所涉及固定液和脱蜡试剂均有一定危险性，建议通风橱内操作。
2. 每切片据组织面积计算试剂用量，一般 100uL/cm^2，培养板内试剂添加参照培养基体积的一半。
3. 甲醛、戊二醛等交联型固定液能稳定组织结构但也会一定程度掩蔽抗原结合位点，因此建议对常规固定样本进行抗原修复。如使用乙醇丙酮等固定可省略。
4. 完整的膜结构会阻碍抗体和荧光染料的进出，因此对于冰冻切片和细胞样本建议通透处理。
5. 不同的荧光信号放大试剂盒请按照对应建议波长进行操作。
6. 与荧光二抗相比，酪酰胺信号放大试剂盒能显示出更高的灵敏度和信号强度。因此，实验时可以适当降低一抗浓度来减少非特异性结合带来的背景荧光，我们建议设置一抗浓度梯度以找到最佳稀释比。
7. 如需考虑背景荧光，建议设置未与一抗孵育的阴性对照。确保该阴性对照在孵育和洗涤过程中没有被阳性样品中的试剂交叉污染。对于组织样品，我们还建议对未染色的对照（不添加抗体或酪酰胺）进行成像，以确定组织自发荧光对背景的影响。
8. 较高的酪胺底物浓度可能会导致信号过强或背景高，可以从 1:5:200 到 1:5:1000 摸索来获取最佳成像效果。
9. 可以在每个酪酰胺反应后通过进行 HRP 淬灭或抗体剥离，依次使用多个酪酰胺扩增试剂盒来标记同一样品上的不同靶标。
10. 为了您的安全和健康，请穿实验服并戴一次性手套操作。
11. 本产品仅供科研使用。请勿用于医药、临床医疗、食品及化妆品用途。

Tyramine Signal Amplification Kit, with HRP-Goat anti-Rabbit IgG and TYR-Cy5 V02

Cat: G4867
Size: 100T/300T
Storage: -20℃, avoid light, valid for 1 year. Period after opening is 3 months.

Kit Components

Reagent		100T	300T	Storage
Reagent(A): Tyr-label Working Solution	Reagent(A1):TYR-Cy5 Solution 500×	20uL	60uL	-20℃, avoid light
	Reagent(A2):Enhancement Solution 100×	0.1mL	0.3mL	2-8℃, avoid light
	Reagent(A3):Diluent Solution	10mL	30mL	2-8℃
Mix A1: A2: A3 in the ratio of 1:5:500 to prepare Tyr-label Working Solution, and use it now.				
Reagent(B):Peroxidase Enhanced Blocking Solution		10mL	30ml	RT
Reagent(C):HRP-Goat anti-Rabbit IgG 100×		0.1mL	0.3mL	-20℃, avoid light

Introduction

The tyramide signal amplification (TSA) system can be used to detect low abundance targets in fluorescence immunocytochemistry (ICC), immunohistochemistry (IHC), and in situ hybridization (ISH) techniques, which can increase signal sensitivity by 100 times. The TSA fluorescence kit uses horseradish peroxidase (HRP) to directly catalyze the covalent deposition of fluorescent groups around the immobilized enzyme. The labeling process is rapid (less than 10 min), and the deposition labeling can be directly observed under a standard or confocal microscope. The TSA kit can be combined with traditional staining methods for multicolor imaging, or more tyramine reactions can be sequentially performed to label different targets on a sample.

The Solarbio Tyramide Signal Amplification (TSA) series products includes 8 kits: the G4860/G4861 is fluorescein labeled and detected at 494nm/ 517nm; The G4862/G4863 was fluorescently labeled as TRAMA and detected at 552nm/ 578nm; The G4864/G4865 was fluorescently labeled as Cyanine3 and detected at 550nm/ 570nm; The G4866/G4867 was fluorescently labeled as Cyanine5 and detected at 648nm/667nm.

Self Provided Materials

1×PBS, 4% PFA, Translucent (Triton X-100, strong), 1×PBST, Sodium Citrate Antigen Repair Solution (1×) or EDTA Antigen Repair Solution, 5% BSA Blocking Solution, or Serum-free Blocking Solution. The above reagents can be self prepared or purchased as commercially available finished reagents.

Protocols*(for reference only)*

Sample Preparation

1. (Optional) Prepare a negative control sample (sample without incubating primary antibody). (See Note 7)
2. Cell sample preparation:
   ① Wash cells with 1×PBS twice for 2 min each time.
   ② After removing PBS, add an appropriate amount of pre-cooled 4% tissue cell fixative and fix at RT for 15 min. (See Note 1)
   ③ Wash cells with 1×PBS twice for 2 min each time.
3. Preparation of paraffin sections:
   ① Place the paraffin slices in a 60 ℃ oven for 30 min.
   ② Soak in xylene or Tissue Transparent Dewaxing Solution, Benzene Free twice at RT for 5 min each time to thoroughly dewaxing.
   ③ At RT, the series of ethanol rehydration:100% ethanol I, 100% ethanol II, 95% ethanol, 90% ethanol, 80% ethanol, 70% ethanol, and distilled water for 5 min each.Soak in 1×PBS for 3 min.
4. Preparation of frozen sections:
   ① Remove the frozen slices from the refrigerator and place them in a 2-8 ℃ refrigerator for 10 min to balance. Wash sections with 1×PBS twice for 2 min each time.
   ② After absorbing PBS, fix with pre-cooled 4% PFA and fix at RT for 15 min. (See Note 2)
   ③ Wash the sample twice with 1×PBS for 2 min each time.
5. Use a pap pen to outline the sample for subsequent localization and imaging.

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Repair and Closure

1. (Optional) Antigen repair: Heat the antigen repair solution adapted to the first antibody to boiling, place the prepared tablets in buffer, and intermittently boil for 10 min. After antigen repair, place the repair solution together at RT and slowly cool down. (See Note 3)
2. (Optional) Cell permeability: Add an appropriate amount of permeability agent (Triton X-100, strong) to the sample and incubate at RT for 10 min. (See Note 4)
3. (Optional) Peroxidase inactivation: Add an appropriate amount of reagent to each sample (B): Peroxidase Enhanced Blocking Solution at RT for 5-10 min, wash cells with 1×PBS twice for 2 min each time.
4. Site blocking: Cover the sample at RT and block it for 10min using serum or BSA or serum-free blocking solution.

Immuno Labeling

1. Dilute the first antibody with sealing solution, dropwise coverage sample and incubate at 37 ℃ for 1 h or 2-8 ℃ overnight (8-12 h).
2. Wash with 1×PBS at RT 3 times for 5 min each time.
3. Dilute the Reagent(C):HRP-Goat anti-Rabbit IgG 100× with blocking solution, dropwise coverage sample and incubate at 37 ℃ for 40 min.
4. Wash with 1×PBS at RT 3 times for 5 min each time.
5. Prepare 100μL-300μL Tyr-label Working Solution for each sample. The working fluid is stored at RT in a dark place and can be stored overnight at most.
6. Cover the sample with dropwise incubation solution and incubate it in dark at RT for 10 min.
7. Wash with 1×PBS at RT 3 times for 5 min each time.
8. Microscopic imaging. For tissue samples on glass slides, please use water-based gelatin sealing agent or anti fluorescence attenuation sealing agent before microscopic imaging.

Note

1. The fixed solution and dewaxing reagents involved in the operation steps have certain risks, and it is recommended to operate in a fume hood.
2. Calculate the reagent dosage for each slice based on the tissue area, usually 100uL/cm^2, and add half of the reference medium volume to the culture plate.
3. Cross linked fixatives such as formaldehyde and glutaraldehyde can stabilize tissue structure but also mask antigen binding sites to a certain extent. Therefore, it is recommended to perform antigen repair on conventional fixed samples. If ethanol acetone is used for fixation, it can be omitted.
4. A complete membrane structure can hinder the entry and exit of antibodies and fluorescent dyes, so it is recommended to conduct transparent treatment for frozen sections and cell samples.
5. Please operate different fluorescence signal amplification kits according to the recommended wavelength.
6. Compared with fluorescent secondary antibodies, the tyramide signal amplification kit can display higher sensitivity and signal intensity. Therefore, during the experiment, the concentration of the primary antibody can be appropriately reduced to reduce the background fluorescence caused by non-specific binding. We suggest setting a concentration gradient of the primary antibody to find the optimal dilution ratio.
7. If background fluorescence needs to be considered, it is recommended to set a negative control that has not been incubated with the first antibody. Ensure that the negative control is not cross contaminated by reagents in the positive sample during incubation and washing processes. For tissue samples, we also recommend imaging the unstained control (without adding antibodies or tyramides) to determine the effect of tissue spontaneous fluorescence on the background.
8. A high concentration of tyramine substrate may lead to strong signal or high background, and the best imaging effect can be obtained by searching from 1:5:200 to 1:5:1000.
9. Multiple tyramide amplification kits can be used in sequence to label different targets on the same sample by HRP quenching or antibody stripping after each tyramide reaction.
10. For your safety and health, please wear laboratory clothes and disposable gloves for operation.
11. This product is for scientific research purposes only. Do not use for medical, clinical, food, or cosmetic purposes.