V02

HRP 免疫双标检测试剂盒(棕红双色)

货号：DA1018
规格：3×30mL
保存：2-8℃, 避光保存, 有效期 1 年。

产品组成：

试剂名称		3×30mL	保存
试剂(A):一标显色工作液	试剂(A1):显色 A 液	1.5mL	2-8℃，避光
	试剂(A2):显色 B 液	1.5mL	2-8℃，避光
	试剂(A3):稀释液	30mL	2-8℃
临用前按照 1:1:18 的比例混匀即为一标显色工作液。			
试剂(B):淬灭修复溶液（10×）		30mL	室温
临用前使用蒸馏水按照 1:9 的比例稀释混匀即为淬灭修复工作液。			
试剂(C):二标显色工作液	试剂(C1):显色 A 液	1.5mL	2-8℃，避光
	试剂(C2):显色 B 液	1.5mL	2-8℃，避光
	试剂(C3):稀释液	30mL	2-8℃
临用前按照 1:1:18 的比例混匀即为二标显色工作液。			

产品介绍：

DAB 显色试剂盒是一种借助辣根过氧化物酶(HRP)反应，可用于免疫组化显色、原位杂交显色或 Western、Southern 、Northern、EMSA 等膜显色的试剂盒。DAB、AEC 等是辣根过氧化物酶的常用底物。在辣根过氧化物酶的催化下，常规 DAB 会产生棕色沉淀，AEC 会产生红色沉淀，使用含特殊增强剂的 DAB 能够产生紫蓝色到蓝黑色沉淀。上述沉淀均不溶于水，AEC 染色沉淀易溶于醇，可用水性封片剂封片，因此可以用于光学显微镜观察的双抗体联合染色。

本产品采用特殊配方，灵敏度高，背景低，重复性好，储存稳定，使用方便，适合于蛋白印迹、免疫组织化学和免疫细胞化学、斑点印迹和生物芯片等的染色和显色反应。

操作步骤：(*仅供参考*)

1、 对于组织切片或蛋白质印记膜，在与辣根过氧化物酶(HRP)标记的抗体或其它形式的 HRP 标记探针孵育后，用适当洗涤液洗涤 3-5 次，每次 3-5 分钟。(见注意事项 1)
2、 将试剂 A1、A2、A3 按照 1:1:18 的比例混合即为一标显色工作液，建议 4h 内使用。(见注意事项 2)
3、 向组织切片或膜上加入适量一标显色工作液，确保能充分覆盖样品。(见注意事项 3)
4、 室温避光孵育 1-30 分钟，显色时间过长可引起本底增高，故应密切观察显色过程(一般 3-10 分钟最理想)，并在本底较浅且达到适当显色强度时以流水漂洗终止显色反应。
5、 1×PBS 润洗 3 次，每次 1min，将切片浸于稀释好的试剂(B):淬灭修复溶液中，热修复。(沸水浴 5-10min 或高压锅开锅后 3-5min。)
6、 短暂封闭(使用一标封闭液，封闭时间减半)后，正常进行第二种一抗孵育和标记二抗孵育。
7、 将试剂 C1、C2、C3 按照 1:1:18 的比例混合即为二标显色工作液，建议 4h 内使用。(见注意事项 2)
8、 向组织切片或膜上加入适量二标显色工作液，确保能充分覆盖样品。(见注意事项 3)
9、 室温避光孵育 1-30 分钟，显色时间过长可引起本底增高，故应密切观察显色过程(一般 3-10 分钟最理想)，并在本底较浅且达到适当显色强度时以流水漂洗终止显色反应。
10、 对于组织切片或细胞样品，显色反应终止后可对其进行其他染料(常用无醇苏木素或甲基绿染色液)复染。对于膜，显色反应终止后，可以室温晾干避光保存。

染色结果：

一标	棕黄色
二标	红色
细胞核	蓝色（苏木素）或绿色(甲基绿)

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注意事项：

1、 洗涤液可使用 1×PBS、1×PBST 及其他免疫组化或膜显色中常用清洗缓冲液。

2、 试剂 A1 和 C1 应为淡黄色至紫红色透明液体，新配的一标显色工作液应为淡红色到淡棕色，二标显色工作液为无色到淡黄色，如颜色过深或出现沉淀，请勿使用。如配好的工作液放置时间较长导致变色或出现沉淀，请勿使用。

3、 通常建议每块组织切片添加 80-100ul 显色工作液，每平方厘米印记膜添加 40-50ul 显色工作液，即 3×30mL 规格的 HRP 免疫双标检测试剂盒(棕红双色)可用于约 300 张组织切片染色或 600 平方厘米的印记膜染色。

4、 试剂(B):淬灭修复溶液(10×)稀释后可循环使用 2-3 次，如需大量使用可使用 C1031-柠檬酸钠抗原修复液(1×)代替。

5、 DAB 有潜在致突变作用，为了您的安全和健康，请穿实验服并戴一次性手套操作。

6、 如显色不佳或长期不用无法确认试剂状态，可取稀释好的二抗孵育液和显色工作液按照 1：3 混匀，静置观察，混合液应在 2min 内变红色，10min 内沉淀。

相关产品：

C1031 柠檬酸钠抗原修复液（1×）
P1032 20×PBS， PH7.2-7.4
G1652 甲基绿染色液(MG,1%)
DA1010 DAB显色试剂盒(20×)
DA1015 DAB显色试剂盒（金属增强法）
DA1016 增强型DAB显色试剂盒(20×)
A2010 AEC底物显色试剂

注：更多使用本产品的文献请参考索莱宝官网。

V02

HRP Double Label Detection Kit (Brown-Red)

Cat: DA1018
Size: 3×30mL
Storage: 2-8℃, avoid light, valid for 1 year.

Kit Components

Reagent		3×30mL	Storage
Reagent (A): First Label Stain Working Solution	Reagent (A1):Color Solution A	1.5mL	2-8℃, avoid light
	Reagent (A2):Color Solution B	1.5mL	2-8℃, avoid light
	Reagent (A3):Color Diluent	30mL	2-8℃
Before use, mix A1,A2,A3 well by1：1：18 to form Reagent (A):First Label Stain Working Solution			
Reagent (B):Quench and Repair Solution(10×)		30mL	RT
Dilute and mix with distilled water by 1:9 before use to obtain the Quenching Repair Working Solution.			
Reagent (C): Second Label Stain Working Solution	Reagent (C1):Color Solution A	1.5mL	2-8℃, avoid light
	Reagent (C2):Color Solution B	1.5mL	2-8℃, avoid light
	Reagent (C3):Color Diluent	30mL	2-8℃
Before use, mix C1,C2,C3 well by1：1：18 to form Reagent (C):Second Label Stain Working Solution			

Introduction

DAB Chromogenic kit is a kind of reagent kit for immunohistochemical chromogenic, in situ hybridization chromogenic or Western, Southern, Northern, EMSA and other membrane chromogenic with the help of Horseradish peroxidase (HRP). DAB, AEC, etc. are commonly used substrates for horseradish peroxidase. Under the catalysis of horseradish peroxidase, conventional DAB produces brown precipitates, AEC produces red precipitates, and using DAB with special enhancers can produce purple blue to blue black precipitates. The above precipitates are insoluble in water, and AEC staining precipitates are easily soluble in alcohol, which can be sealed with water-based sealing agents. Therefore, they can be used for dual antibody combined staining observed under optical microscopy.

This product adopts a special formula with high sensitivity, low background, good repeatability, stable storage, and convenient use. It is suitable for staining and color reaction in protein blotting, immunohistochemistry, immunocytochemistry, dot blotting, and biochip.

Protocols (*only for reference*)

1. For tissue sections or protein imprinting membranes, after incubation with horseradish peroxidase (HRP) labeled antibodies or other forms of HRP labeled probes, wash them 3-5 times with appropriate washing solution, each time for 3-5 min. (See Note 1)
2. Mix reagents A1, A2, and A3 well by 1:1:18 to form First Label Stain Working Solution. It is recommended to use within 4 h. (See Note 2)
3. Add an appropriate amount of First Label Stain Working Solution to the tissue slice or membrane to ensure sufficient coverage of the sample. (See Note 3)
4. Incubate at room temperature in dark for 1-30 min. If the color development time is too long, it can cause an increase in background. Therefore, the color development process should be closely observed (usually 3-10 min is optimal), and the color development reaction should be terminated by rinsing with running water when the background is shallow and reaches the appropriate color development intensity.
5. Rinse the slices with 1 × PBS three times for 1 min each time, immerse them in diluted Reagent (B):Quench and Repair Solution, and heat repair them. (Boiling water bath for 5-10 min or pressure cooker for 3-5 min after boiling.)
6. After a brief closure (using a standard blocking solution, the closure time is halved), the second type of first antibody incubation and labeled second antibody incubation are carried out normally.
7. Mix the reagents C1, C2, and C3 evenly by 1:1:18 to form Second Label Stain Working Solution. It is recommended to use it within 4 h. (See Note 2)
8. Add an appropriate amount of Second Label Stain Working Solution to the tissue slice or membrane to ensure sufficient coverage of the sample. (See Note 3)
9. Incubate at room temperature in dark for 1-30 min. If the color development time is too long, it can cause an

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increase in background. Therefore, the color development process should be closely observed (usually 3-10 min is optimal), and the color development reaction should be terminated by rinsing with running water when the background is shallow and reaches the appropriate color development intensity.

10. For tissue sections or cell samples, after the color reaction is terminated, other dyes (commonly used no alcohol hematoxylin or methyl green staining solution) can be used for re staining. For the film, after the color reaction is terminated, it can be dried at room temperature and stored away from light.

Result

One label	Brown Yellow
Second Label	Red
Cell Nucleus	Blue (hematoxylin) or Green (methyl green)

Note

1. Rinsing solution can use 1×PBS, 1×PBST and other commonly used washing buffers in IHC or membrane chromogenic.
2. Reagent A1 and C1 shall be light yellow to purple transparent liquid, the new first standard color working solution shall be light red to light brown, the second standard color working solution shall be colorless to light yellow, if too dark or precipitation, do not use. If the good working solution is placed for a long time, resulting in discoloration or precipitation, do not use it.
3. It is usually recommended to add 80-100ul Stain Working Solution to each tissue, and 40-50ul Stain Working Solution to each square centimeter of imprinting film. It is that a 3×30mL HRP Double Label Detection Kit (Brown-Red) can be used for staining approximately 300 tissue sections or staining imprinted membranes with an area of 600 square centimeters.
4. Reagent (B):Quench and Repair Solution(10×) can be reused 2-3 times after dilution. If a large amount of use is required, C1031 sodium citrate antigen repair solution (1) can be used ×) Replace.
5. DAB has potential mutagenic effect. For your safety and health, please wear White coat and disposable gloves.
6. If the chromogenic reaction is poor or cannot confirm the reagent quality for long time, can mix diluted secondary antibody incubation solution and Stain Working Solution in a ratio of 1:3, and left to stand for observation. The mixture should turn brownish yellow or dark blue within 2 min and precipitate within 10 min.