

# Villin 免疫组化检测试剂盒

## 【产品货号】

IHCM6222

## 【产品名称】

- 1、通用名称: Villin mouse mAb ABT097 免疫组化检测试剂盒
- 2、英文名称: Villin High Sensitive and Rapid Immunohistochemical Kit

## 【主要组成成份】

编号	名称	规格	保存	
试剂 9A	脱蜡修复二合一溶液 20X	60 mL	2~8° C	
试剂 B	过氧化物酶封闭缓冲液	3 mL	2~8° C	
试剂 C	HRP 多聚合抗兔/鼠二抗	3 mL	2~8° C	
试剂 D	超敏 DAB 浓缩液 20X	150 µ L	2~8° C	
试剂 E	超敏 DAB 缓冲液	3 mL	2~8° C	
试剂 F	苏木精染色缓冲液	3 mL	2~8° C	
试剂 G	即用型抗体溶液	3 mL	2~8° C	

#### 【预期用途】

免疫组织化学染色。

## 【作用原理】

#### 【包装规格】

3mL 装。标准可染色 30 张切片。

#### 【储存条件及有效期】

- 1、储存要求: 2℃~8℃密封保存。
- 2、有效期:一年。

#### 【适用仪器】

手工操作

#### 【自备材料】

1、合适的加热装置,染缸,移液器,盖玻 片等常用耗材

- 2、 无水酒精
- 3、中性树胶
- 4、PBS、纯水等常用试剂

## 【样本要求】

新鲜活检或外科样本组织,甲醛固定,固定时间8-24小时要求取材、脱水、石蜡包埋并制成蜡块。配合 Immunoway 常规浓缩型或即用型一抗使用。

#### 【阳性对照】

Appendix /\_colon

## 【染色部位】

Membranous, Cytoplasmic

#### 【使用前准备】

1、使用去离子水稀释试剂 9A, 试剂 D 至工作液。

试剂 9A, 试剂 D 均为 20 倍浓缩液, 按照体积比 1:19 加入去离子水

2、各试剂使用前在使用前室温放置 5min。

## 【使用步骤】

- 1. 将试剂 9A (脱蜡修复二合一) 工作液放入 修复盒中, 加热至沸腾。
- 2. 将切片放入沸腾的试剂 9A (脱蜡修复二合一) 工作液中(为保证修复液的pH值,不能使用金属切片架),液体完全浸没切片上的组织。中小火持续加热 30 min.
- 3. 将修复盒撤离加热源,自然冷却至室温。
- 4. 取出组织切片放入装有蒸馏水的烧杯中, 再用蒸馏水浸洗 5-6 遍.
- 5. 将玻片沥干几秒钟,用滤纸把组织周围的水分擦掉后,滴加试剂 B (过氧化物酶封闭缓冲液),室温孵育 15 min, PBS 冲洗 2 min × 3 次。
- 6. 将玻片沥干几秒钟,用滤纸把组织周围的水分擦掉后,用免疫组化笔将组织圈起来(首尾要闭合,且不能画到组织上),滴加一抗到组织上,直至完全覆盖组织。室温或37°C 孵育45-60分钟,或4°C 湿盒中过夜(后37°C 复温30 min),PBS 冲洗2 min ×3次。
- 7. 将玻片沥干几秒钟,用滤纸把组织周围的水分擦掉后,滴加试剂 C ( HRP 多聚合抗兔/鼠二抗),室温孵育 30 min,PBS 冲洗 2 min×3 次。
- 8. 每 1 mL 的试剂 E 超敏 DAB 底物液中,加入



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1 滴(约50 μL)的试剂 D超敏 DAB 浓缩液。混合均匀,即配成 DAB 工作液。此溶液必须现配现用,配好后避光保存。4 小时内使用,剩余的液体舍弃。

- 9. 擦去组织周边多余水分,滴加 DAB 显色剂,在显微镜下观察,控制好 DAB 显色时间,阳性信号为棕黄色或褐黄色,切勿显色过深,用纯净水冲洗切片终止显色。
- 10. 擦去组织周边多余水分,滴加试剂F苏木素染色液,染色 5min,纯净水冲洗。
- 11. 使用碱水或 1%盐酸酒精溶液返蓝 1min 后 洗净或自来水冲洗  $5^{\circ}10$  min。
- 12. 85% 95% 100%酒精脱水,每次 3min,透明剂透明,滴加中性树胶后封片,晾干。

#### 【产品性能指标】

- 1、符合性: 阳性对照(实验自备)结果为阳性,阳性着色的定位应准确,无背景着色;空白对照和阴性对照染色结果为阴性。
- 2、批内重复性:同一组织来源的组织切片染色的强度和定位无明显差异。
- 3、批间重复性:不同批号试剂对同一组织来

源的组织片染色的强度和定位无明显差异。

#### 【注意事项】

- 1、本品仅用于科研,不做其他用途。
- 2、需专业人员使用。
- 3、应用适当防护措施,避免试剂同皮肤和眼睛接触。
- 4、废液处理: 进行无害化处理,并符合相关的环保要求。
- 5、操作过程中需保持载玻片组织的湿润,如出现干片情况,会导致非特异性的染色结果。6、. 持续加热修复的过程中,只需中小火维持沸腾,切勿高火加热使脱蜡修复试剂溅出烧杯。
- 7、. 配置和使用 DAB 的过程中应做好防护, 以避免试剂同皮肤与眼睛接触。

## 【常见问题】

- 1、染色过深:一抗浓度过高,时间过长。
- 2、染色过浅或无染色:一抗浓度过低,时间过短。
- 3、无特异性染色:切片脱蜡不彻底。可适当延长烤片时间。

## 【图标示意】

以下图标可能部分标注于产品外标签上





## Villin mouse mAb ABT097 High Sensitive and Rapid

## Immunohistochemical Kit

Catalog No.: IHCM6222

Size: 3ml

Immunohistochemical staining, combined with rabbit or mouse primary antibody.

Please read the provided manual as suggested experimental protocols may have changed.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.

#### Introduction

High Sensitive and Rapid Immunohistochemical Kit, don't need to prepare for fume cupboard and traditional dewaxing equipment. Integrate the traditional xylene dewaxing three times, gradient ethanol hydration three to five times and antigen repair into one solution, which effectively reduce the operation <u>procedure</u> and improve the stability of dyeing.

ASSAY FORMAT

#### MATERIALS INCLUDED

编号	Item	Quantity	Storage Condition
reagent 9A	20x Dewaxing and repair two-in-one solution	60 mL	2~8° C
reagent B	Peroxidase seals buffer	3 mL	2~8° C
reagent C	anti Rabbit/Mouse polymer HRP secondary antibody	3 mL	2~8° C
reagent D	20xDAB high sensitive buffer	150 µ L	2~8° C
reagent E	1xDAB high sensitive buffer	3 mL	2~8° C
reagent F	Hematoxylin dyeing buffer	3 mL	2~8° C
reagent G	Antibody buffer	3 mL	2~8° C

#### ADDITIONAL MATERIALS REQUIRED

1 Heating dye VAT, pipette, repair tank, cover glass

2 absolute alcohol

3 Rhamsan gum

4 PBS



#### STORAGE INFORMATION

1 storage requirements: 2°C ~8°C sealed storage.

2 Unopened Kits: Store at 2°C ~ 8°C for 1 year.

#### SAMPLE PREPARATION

Paraffin tissue section

#### Preparation

- 1. Dilute the 20x reagent A and D to 1x using ddH<sub>2</sub>O.
- 2. Each reagent should be placed at room temperature for 5min before use.

#### **Protocol**

- 1. Place reagent 9A working solution in the repair tank and bring to A boil.
- 2. Sections are placed in boiling reagent A working solution (Metal slicing rack cannot be used, in order to ensure the pH value of the repair solution), and the liquid completely immerses the tissue. Keep heating for 30 min with small and medium fire.
- 3. Remove the repair tank from the heating source, and natural cooling to room temperature.
- 4. Remove the section and put them into a beaker filled with ddH2O, then soak them in distilled water for 5-6 times.
- 5. Drain slides for a few seconds and wipe around the sections with filter paper, apply reagent B ,incubate 15min at room temperature , rinse 3 x 2min with PBS.
- 6. Drain slides for a few seconds and wipe around the sections with filter paper, the tissue was enclosed with an immunohistochemical pen, dilute the primary antibody ,apply the primary antibody to the tissue, incubate 45-60min at room temperature or  $37^{\circ}$ C, or overnight at  $4^{\circ}$  C (rewarming 30 min at  $37^{\circ}$ C) in a wet box, rinse 3 x 2min with PBS.
- 7. Drain slides for a few seconds and wipe around the sections with filter paper, apply reagent C, incubate 30 min at room temperature ,rinse 3 x 2min with PBS.
- 8. Add 1 drop (about 50  $\,^{\,}$  L) of reagent D into concentrated DAB substrate solution for every 1 mL of reagent E. DAB working liquid is prepared by mixing evenly. The solution must be prepared and used now, and stored away from light after preparation. Use within 4 hours, discard the remaining liquid.
- 9. Wipe off the excess water around the tissues, drop DAB chromogenic agent, observe under the microscope, control the DAB chromogenic time, the positive signal is brownish yellow or brownish yellow, do not overcolor, rinse the slices with pure water to terminate chromogenic.
- 10. Wipe off the excess water around the tissues, drop the reagent F hematoxylin staining solution, stain for 5min, and rinse with pure water.
- 11. Use alkaline water or 1% hydrochloric acid alcohol solution to turn blue for 1min and then wash it or wash it with running water for 5-10 min.
- 12. 85%, 95% and 100% alcohol were dehydrated for 3min each time. The transparent agent was transparent.

#### Property

- 1. Conformity: the positive control (prepared by the experiment) results are positive, and the location of positive coloring should be accurate without background coloring; The staining results of blank control and negative control were negative.
- 2. Intra-batch repeatability: there was no significant difference in staining intensity and location of tissue sections from the same tissue source.
- 3. Repeatability between batches: there was no significant difference in staining intensity and positioning of tissue slices from the same tissue source with different batches of reagents.

#### Notes

- 1. This product is only used for scientific research, not for other purposes.
- 2. It needs to be used by professionals.
- 3. Apply appropriate protective measures to avoid contact with skin and eyes.



- 4, waste liquid treatment: harmless treatment, and in line with the relevant environmental requirements.
- 5. During the operation, the tissue of the slide should be kept moist. If the slide is dry, non-specific staining results will be caused.
- 6. In the process of continuous heating and repair, only small and medium fire is needed to maintain boiling, and do not heat with high fire to make the dewaxing repair reagent splash out of the beaker.
- 7. Precautions should be taken during the configuration and use of DAB to avoid contact between reagents and skin and eyes.