# Wilms' Tumor 1(WT1)免疫组化检测试剂盒

**【产品货号】**
IHCM6533

**【产品名称】**
1、通用名称：Wilms' Tumor 1(WT1) mouse mAb ABT-WT1 免疫组化检测试剂盒
2、英文名称：Wilms' Tumor 1(WT1) 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 | 150μL | 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】**
超敏快速免疫组化检测试剂盒，摆脱传统免疫组化实验条件对实验的限制和束缚，无需通风厨和传统的脱蜡修复缸，将传统的三次二甲苯脱蜡、三到五次梯度乙醇水化和抗原修复整合成一个溶液，有效的缩短了操作时间，简化了繁琐的操作步骤，同时，减少了操作中的变量，提高了染色的稳定性。脱蜡抗原修复二合一试剂应用新的环保技术，不仅把对身体的危害降到了最低，而且对环境不会造成任何污染。免疫组化实验用到的试剂一站式配齐，具有高灵敏度的 HRP 多聚合物二抗(Anti-Rabbit/Mouse)和DAB显色液配套使用，使得该试剂盒具有操作方便、快速、灵敏度高等特点。

**【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】**
Fallopian tube / kidney

**【染色部位】**
Nuclear

**【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 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 5~10 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

货号

批号

仅供体外诊断用

仅供科研使用


保存温度


参照使用说明书


制造厂商


有效期

# Wilms' Tumor 1(WT1) mouse mAb ABT-WT1 High Sensitive and Rapid Immunohistochemical Kit

## Catalog No. : IHCM6533

## 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 procedure 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 | 150μL | 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℃ ~ 8℃ sealed storage.

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

## SAMPLE PREPARATION

Paraffin tissue section

### Preparation

1. Dilute the 20x reagent A and D to 1x using dd$H_2O$.

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℃, or overnight at 4° C (rewarming 30 min at 37℃ ) 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 μL) 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.