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Rat Peripherin Ready-To-Use IHC Kit

Cat. No.: IHC0108R

Sample Type: FFPE tissue **Size: 50T (including a control slide)**

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

General Information

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Rat Peripherin Rabbit pAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Rat colon)	1 slide	RTU	RT
12	Datasheet	1 copy		

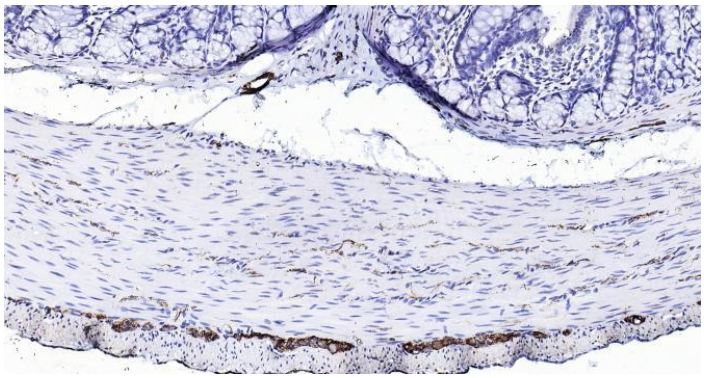
Background

Peripherin is a 57kD type III intermediate filament that is a specific marker for peripheral neurons, including enteric ganglion cells. Peripherin is expressed in the developing peripheral nervous system and is highly enriched in neuronal derivatives of the neural crest. Peripherin offers an advantage over other neural markers, such as S100 and NSE in that it does not stain chrmaffin cell types.

Synonyms

NEF 4; NEF4; NEF-4; Neurofilament 4; PRPH; PRPH1; PRPH 1; NEF 4; Neurofilament 4 (57kD); Perf; PERI_HUMAN.

Validation Data



Immunohistochemical analysis of paraffin embedded rat colon tissue slide using IHC0108R (Rat Peripherin IHC Kit).

Immunohistochemistry Protocol

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval

Add 100×**Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Rat Peripherin Rabbit pAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. Dehydration Sheet

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0108R, Bioss Antibodies". Citation example: "Rat tissue sections using Rat Peripherin IHC Kit (IHC0108R, Bioss Antibodies) were stained for Peripherin according to the manufacturer's instructions.

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Rat Peripherin Ready-To-Use IHC Kit

大鼠外周蛋白即用型免疫组化试剂盒

产品货号：IHC0108R
样本类型：FFPE 组织切片 **产品规格：50T （包含一个对照切片）**
保存条件：见下表。有效期 6 个月。

产品组分及规格

编号	组分	规格	浓度	储存
1	PBS 缓冲液（干粉）	2 L×2	20x	室温
2	抗原修复缓冲液	20 ml	100x	2-8℃
3	内源性过氧化物酶阻断剂	3 ml	RTU	2-8℃
4	封闭工作液	3 ml	RTU	2-8℃
5	一抗（Rat Peripherin Rabbit pAb）	6 ml	RTU	2-8℃
6	二抗（HRP-Goat anti-Rabbit IgG pAb）	6 ml	RTU	2-8℃
7	DAB kit（20×）显色液	0.3 ml	RTU	-20℃
8	DAB kit（20×）稀释液	0.3 ml	RTU	-20℃
9	复染试剂	5 ml	RTU	室温
10	封片剂	5 ml	RTU	室温
11	对照切片（大鼠结肠）	1 张	RTU	室温
12	说明书	1 份		

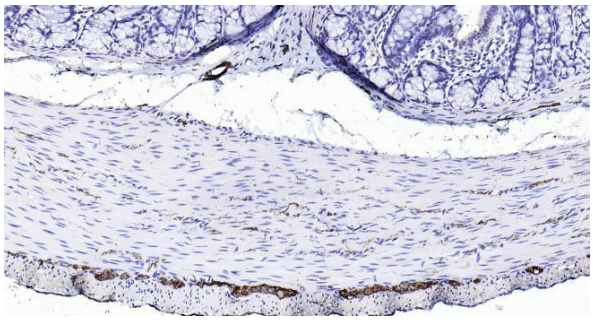
背景

Peripherin is a 57kD type III intermediate filament that is a specific marker for peripheral neurons, including enteric ganglion cells. Peripherin is expressed in the developing peripheral nervous system and is highly enriched in neuronal derivatives of the neural crest. Peripherin offers an advantage over other neural markers, such as S100 and NSE in that it does not stain chrmaffin cell types.

别名

NEF 4; NEF4; NEF-4; Neurofilament 4; PRPH; PRPH1; PRPH 1; NEF 4; Neurofilament 4 (57kD); Perf; PERI_HUMAN.

验证数据



使用 IHC0108R（大鼠外周蛋白 IHC 试剂盒）对石蜡包埋的大鼠结肠组织切片进行免疫组化分析。

石蜡包埋组织的免疫组织化学方案

1. 脱蜡水化

石蜡切片置于新鲜二甲苯中浸泡脱蜡 3 次，每次 15 min；依次置于不同浓度（100%、95%、90%、80%、70%）乙醇浸泡各 5 min，再置于蒸馏水洗涤 5 min，重复 3 次。

2. 抗原修复

沸水浴修复：将 100×**抗原修复缓冲液（试剂 2）**用蒸馏水稀释成 1×抗原修复缓冲液，放入修复盒中并提前加热至 95-100℃（注意盖好以防液体蒸发），然后将切片放入修复盒中，在沸水浴环境中保持外沸状态 15 min，室温自然冷却；用 **PBS 缓冲液（试剂 1**，将干粉溶解在 2L 蒸馏水中）清洗 5 min，重复 3 次。

3. 阻断内源性过氧化物酶

用吸水纸吸去玻片上多余的液体，用免疫组化笔在组织周围画圈，加入 2-4 滴**内源性过氧化物酶阻断剂（试剂 3）**，室温下置于湿盒中孵育 15 min，用 PBS 洗涤 5 min，重复 3 次。

4. 血清封闭

用吸水纸吸去玻片上多余的液体，加入 2-4 滴**封闭工作液（试剂 4）**，置于湿盒内 37℃封闭 20 min，以减少非特异性染色。

5. 一抗孵育

用吸水纸吸去玻片上多余的液体，加入 2-4 滴**大鼠 Peripherin 兔多抗工作液（试剂 5）**，置于湿盒中，4℃孵育过夜或 37℃孵育 1-2 h。

6. 复温

4℃孵育过夜后，室温下复温 15 min（若在室温下孵育一抗，则直接进入下一步清洗）；用 PBS 洗涤 5 min，重复 3 次。

7. 二抗孵育

用吸水纸吸玻片上多余的液体，加入 2-4 滴 **HRP 标记羊抗兔 IgG 工作液（试剂 6）**，置于湿盒中，37℃孵育 1-2 h；用 PBS 洗涤 5 min，重复 3 次。

8. 显色

用吸水纸吸去玻片上多余的液体，在每张切片上滴加约 50 μL 新配制的 **DAB 工作液（试剂 7:试剂 8:PBS=1:1:18）**，作用 3-5 min。显微镜下观察结果，达到合适的显色强度后，用蒸馏水冲洗切片以终止反应，用蒸馏水冲洗 5 min，重复 3 次。

9. 复染

滴加适量**复染试剂（试剂 9）**复染 3-5 min，蒸馏水冲洗 5 min，滴加盐酸酒精分化约 30 s，蒸馏水洗涤 5 min，重复 2 次。

10. 脱水封片

将玻片依次置于不同浓度（70%、80%、90%、95%、100%）乙醇，各 5 min；然后置于新鲜二甲苯中浸泡脱蜡 3 次，每次 15 min。用吸水纸吸去多余的二甲苯，滴加适量**封片剂（试剂 10）**在组织上，将盖玻片盖在组织上，避免产生气泡。

注意事项

1. 建议检测时进行阴性及阳性对照，以提高实验的可靠性。
2. 本品中的配套试剂，请不要用其他生产商产品替换使用。
3. DAB 为致癌物质，请采取必要的防范措施。
4. PBS 洗涤液（试剂 1）配制后在 4℃可保存一周；抗原修复液（试剂 2）及显色剂（试剂 7 和 8）的工作液需每次实验时现用现配。

*5. 发表论文时引用本产品的写作建议 "IHC0108R, Bioss Antibodies"。引用示例: “Rat tissue sections using Rat Peripherin IHC Kit (IHC0108R, Bioss Antibodies) were stained for Peripherin according to the manufacturer's instructions.”