PRODUCT-SPECIFIC PROTOCOLS

IHC (For 66241-1-Ig only)

Sample type	Sample preparation	Antigen Retrieval	Blocking buffer	Primary antibody dilution	Incubation time	Signal detection
mouse spleen tissue	Paraffin-embedded	Tris-EDTA buffer (pH 9.0)	5% goat serum in TBS	1:400	1.5 h at room temp	Reagents for color development

Protocol for IHC (English version)

Deparaffinize and rehydrate

1. Deparaffinize slides in 2 baths of xylene for 20 minutes each.
2. Rehydrate slides by sequential incubation with 100%, 95%, 80%, and 60% ethanol, 5 minutes for each bath.
3. Immerse slides with distilled water 3 times for 1 minute each.

Antigen Retrieval (check table above)

1. Transfer slides to a container and cover with antigen retrieval solution according to the table above.
2. Heat slides in a microwave on medium power for 10 minutes.
3. Allow slides to cool in the buffer for 35 minutes.

Incubation with Primary Antibody and Signal Detection

1. Rinse slides 3 times with 1XTBS for 1 minute each.
2. Incubate slides in 2M HCl and at 37℃ for 30 minutes. NOTE:HCl is needed to be ready to use.
3. Immerse slides in distilled water 3 times for 1 minute each.
4. Incubate slides with 3% H_2O_2 solution (diluted in distilled water) for 10 minutes to quench endogenous peroxidase activity at room temperature.
5. Rinse slides 3 times with 1XTBS for 1 minute each.
6. Block the slides at room temperature for 1 hour in Blocking buffer.
7. Incubate slides with primary antibody (diluted in dilution buffer) for 1.5 hours at room temperature.
8. Rinse slides 3 times with 1XTBS for 1 minute each.
9. Incubate slides with sufficient peroxidase labeled polymer secondary antibody for 30 minutes at room temperature.
10. Rinse slides 3 times with 1XTBS for 1 minute each.
11. Incubate slides with enough DAB for 2-5 minutes at room temperature till a brown color develops.
12. Rinse slides 3 times with distilled water for 1 minute each.
13. Incubate slides in a bath of hematoxylin at room temperature to stain the nuclei for 3 minutes.
14. Rinse slides 3 times with 1XTBS for 1 minute each.
15. Immerse slides in distilled water for 5 minutes.
16. Immerse slides sequentially into 60%, 80%, 90%, and 100% ethanol bath for 5 minutes each.
17. Immerse slides in xylene bath for 5 minutes, then immerse in another fresh xylene for 5 minutes.
18. Mount the slides with a small drop of neutral balsam and add a coverslip. Air-dry slides in the hood.
19. Examine slides under a microscope.

Protocol for IHC (Chinese version)

脱蜡和水化

1. 脱蜡：二甲苯处理石蜡包埋的组织切片2 次，每次 20 分钟。
2. 水化：依次用100%、95%、80%和60%的乙醇浸泡切片，每次 5 分钟。
3. 去离子水浸洗切片3 次，每次1 分钟。

抗原修复 (跟检测样本有关,对于该切片我们进行了热修复)

1. 切片转入新容器，浸没于足量的Tris-EDTA 缓冲液（pH 9.0）。
2. 微波炉中等功率加热切片 10 分钟。
3. 切片置缓冲液中室温冷却 35 分钟。

抗体孵育及信号检测

1. 1XTBS 冲洗切片3 次，每次 1 分钟。
2. 将切片浸入装有2M HCl 的容器中，37℃孵育 30分钟。注意：HCl需现配现用。
3. 去离子水冲洗切片3 次，每次1 分钟。
4. 将切片浸入装有3% H_2O_2 溶液（去离子水稀释）的容器中，盖上盒盖，室温密闭孵育 10 分钟，灭活内源性过氧化物酶。
5. 1X TBS 冲洗切片3 次，每次 1 分钟。
6. 3% BSA（1× TBS稀释）室温封闭切片1 小时，勿洗，用吸水纸吸去多余液体。
7. 一抗（66241-1-Ig，BrdU Mouse McAb，1:400）稀释后孵育切片，室温1.5 小时或4 ℃过夜。
8. 1X TBS 冲洗切片3 次，每次1 分钟。
9. 足量的过氧化物酶标记的二抗室温孵育切片 30 分钟。
10. 1X TBS 冲洗切片3 次，每次 1 分钟。
11. 足量的DAB 室温孵育切片 2-5 分钟，至呈现棕色。
12. 去离子水冲洗切片3 次，每次 1 分钟。
13. 苏木精室温孵育切片，染核 3 分钟。
14. 1X TBS 冲洗切片3 次，每次 1 分钟。
15. 去离子水中浸泡切片 5 分钟。
16. 依次用 60%、80%、95%和100% 的乙醇浸泡切片，每次 5 分钟。
17. 二甲苯浸泡切片5 分钟，再换新鲜的二甲苯浸泡 5 分钟。
18. 在切片上滴加适量中性树脂，盖上盖玻片。通风橱中晾干切片。
19. 显微镜下观察切片。