PRODUCT-SPECIFIC PROTOCOLS

IF (For 66241-1-Ig only)

Protocol for IF (English version)

1. BrdU Incorporation: Dilute BrdU in fresh, pre-warmed growth medium to a final concentration of 0.03 mg/mL. Add mixture to cells and incubate at 37℃ for 2 hours.
2. Aspirate media, cover cells completely with 70% ethanol. NOTE:ethanol is pre-cooled at -20℃
3. Allow cells to fix for 5 minutes at room temperature.
4. Aspirate fixative, rinse three times in 1X PBS for 5 minutes each.
5. Add 2M HCl and at 37℃ for 30 minutes. NOTE: HCl is needed to be ready to use.
6. Aspirate HCl and rinse two times in 1X PBS for 5 minutes each.
7. Incubate the samples with Blocking buffer at room temperature for 1 hour.
8. Aspirate Blocking buffer and incubate with primary antibody at room temperature for 2 hours or at 4℃ for overnight.
9. Wash samples with 1XPBS 3 times for 5 minutes each.
10. Incubate with fluorochrome-labeled secondary antibody diluted in Antibody dilution buffer for 1 hour at room temperature in dark.
11. Wash coverslip with 1XPBS 3 times for 5 minutes each.
12. Mount and seal the sample, then visualize the target antigen using a fluorescent microscope.

Protocol for IF (Chinese version)

1. BrdU掺入：在新鲜预热的生长培养基中稀释BrdU，至最终浓度为 0.03 mg/mL。向细胞中加入混合物后在37℃下孵育 2 小时。
2. 吸干培养基后用提前在-20℃预冷的70 %乙醇完全浸没细胞。
3. 室温下固定细胞 5 分钟。
4. 吸去固定液，用 1X PBS 漂洗三次，每次 5 分钟。
5. 37℃下用2M HCl孵育细胞 30 分钟。注意：HCl需现配现用。
6. 吸去HCl后用1X PBS 漂洗两次，每次 5 分钟。
7. 室温下用封闭缓冲液孵育细胞 1 小时。
8. 吸去封闭缓冲液，一抗室温孵育 2 小时或4℃孵育过夜。
9. 1X PBS 漂洗三次，每次 5 分钟。
10. 选择相应的荧光标记二抗，根据抗体说明书推荐稀释比例，用1% BSA-PBS进行稀释，室温下避光孵育 1 小时。
11. 二抗孵育结束后，1X PBS 漂洗三次，每次 5 分钟。
12. 在荧光显微镜下观察染色样本。