Anti- Phospho-Ser503 Kv3.1, Voltage-Gated, Potassium Channel Immunocytofluorescence Protocol

Catalog #: p1550-503
Species: rabbit
Tissue/ Cells: CHO cells transfected with KV 3.1 and treated with PMA

Fixation: 4% paraformaldehyde, 10 minutes at 4C
Antibody incubation: Primary Antibody- 4C, 24 hours Secondary Antibody- 4C, overnight

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in 1xPBS, chilled
- ✓ **1x PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4, pH 7.6
- ✓ **Permeabilization solution (PBST):** 0.5% Triton-X 100 in 1xPBS
- ✓ **Blocking buffer:** 5% FBS (fetal bovine serum) in 1xPBS
- ✓ **Incubation buffer:** 1% FBS in 1xPBS
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa Fluor 488 from Invitrogen, cat # A11008
- ✓ **Mountant:** Citifluor Mountant Media from Ted Pella, Cat # 19470

Before you begin

The CHO (Chinese Hamster ovary) cells were transfected and stably express various Kv3.1 isoforms and mutants. For more information regarding the protein expression and propagation of the cells reference P. Song et al 2006.

Protocol

1. Draw off culture medium with aspirator and wash cells with 1xPBS.
2. Remove 1xPBS and add 1 ml of fixative to the dish. Incubate at 4C for 10 minutes.
3. Remove the fixative and wash with 1xPBS 3 times.
4. Permeabilize cells with permeabilization solution for 10 minutes.
5. Remove permeabilization solution and add blocking buffer. Incubate for 30 minutes at room temperature.
6. Rinse cells with 1xPBS 3 times, in 10 minute intervals.
7. Dilute the Anti- Phospho-Ser503 Kv3.1, Voltage-Gated, Potassium Channel (Cat. # p1550-503) to 1:400 in incubation buffer. Incubate cells at 4C for 24 hours.
8. Remove primary antibody and wash with 1xPBS 3 times, in 10 minute intervals.
9. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells overnight at 4C.

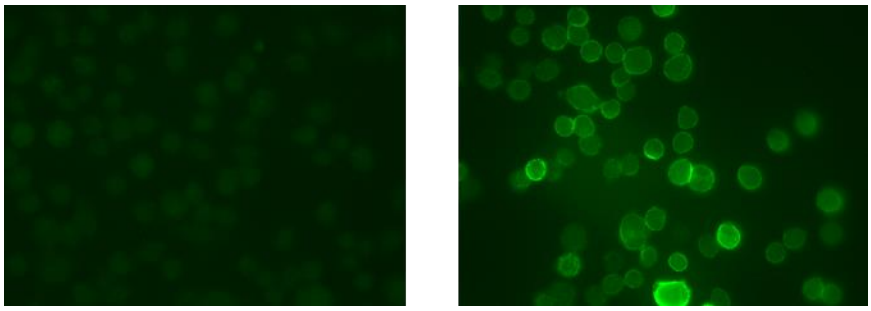
Tech Tip:

a. Goat Anti-Rabbit Alexa Fluor 488 (Thermo Fisher, cat # A11008) diluted 1:700 was used in this protocol.

10. Remove secondary antibody and wash with 1xPBS 3 times, in 5 minute intervals.
11. Apply mounting medium intended for fluorescence onto dish and gently place glass cover slip before viewing under the microscope.

Tech Tip:

a. There are various mounting medias for fluorescence that can be used, for this protocol the medium used was Citifluor AF1 (Ted Pella).



Immunostaining of Chinese Hamster Overy (CHO) cells stably transfected with KV3.1b gene with the phospho-Ser^{503} Kv3.1 subunit antibody (cat # p1550-503, green, 1:400). The image on the right shows cells that have been treated with the protein kinase C activator PMA (500nM) while the control cells are on the left.

Reference:

Song, P. and Kaczmarek, L.K., 2006. Modulation of Kv3. 1b potassium channel phosphorylation in auditory neurons by conventional and novel protein kinase C isozymes. *Journal of Biological Chemistry*, 281(22), pp.15582-15591.