Anti-Phospho-Thr^{202}/Tyr^{204} ERK/MAPK Antibody Immunocytofluorescence Protocol

Catalog #: p160-2024
Species: rabbit
Tissue/ Cells: Human lymphocytes

Fixation: 4% paraformaldehyde, 12 minutes
Antibody incubation: Primary Antibody- 4C, 24 hours Secondary Antibody- 1 hour, RT

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde in 1xPBS
- ✓ **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):** 0.12% Triton-X in PBS
- ✓ **Blocking buffer:** 10% Horse serum in 1xPBS
- ✓ **Incubation buffer:** 1% Horse serum in 1xPBS
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Cy5 AffiniPure from Jackson ImmunoResearch at # 111-175-144
- ✓ **Hoechst stain**
- ✓ **Mountant:** Permount, Fisher Scientific (catalog # SP15-100)

Before you begin

The human blood samples were centrifuged and lymphocytes were isolated and incubated on slides or 24 well plates. For more information regarding the collection, isolation, and propagation of the cells reference Erickson et al, 2017.

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. Fix for 12 minutes.
3. Remove the fixative and wash with 1xPBS 3 times.
4. Permeabilize cells with permeabilization solution for 12 minutes.
5. Remove permeabilization solution and add blocking buffer. Incubate for 15 minutes at room temperature.
6. Rinse cells with 1xPBS 3 times, in 20 minute intervals.
7. Dilute the Anti- Phospho-Thr^{202}/Tyr^{204} ERK/MAPK (Cat. # p160-2024) to 1:250 in incubation buffer. Incubate cells overnight at 4C.
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 at room temperature for 1 hour in the dark.
   Tech Tip:
   a. Goat Anti-Rabbit Cy5 (Jackson ImmunoResearch, cat #111-585-144) diluted 1:200 was used in this protocol.
10. Remove secondary antibody and wash with 1xPBS 3 times, in 5 minute intervals.
11. Dilute Hoechst stain in 1xPBS at 1:500. Stain cells for 2 minutes and remove solution.
12. 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 Permount (Fisher Scientific, Cat# SP15-100).

Reference:

Erickson, C.A., Ray, B., Wink, L.K., Bayon, B.L., Pedapati, E.V., Shaffer, R., Schaefer, T.L. and Lahiri, D.K., 2017. Initial analysis of peripheral lymphocytic extracellular signal related kinase activation in autism. *Journal of Psychiatric Research*, 84, pp.153-160.