ImageStream® Immune Synapse Staining Protocol

Immune Synapse Staining

This protocol is adapted from Wabnitz, Eur J Immunol. 2011 Nov;41(11):3157-69.

Samples: (2 x 10^6 cells per test)

Single fluorescent color control samples – unstained, AF488, PE-TexasRed, AF647, DAPI

Experimental samples – untreated, positive control treatment, experimental treatment

Materials

1. 1x Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)
2. 0.1% triton X-100/2% FBS/PBS (PermWash Buffer): 10% triton stock, Calbiochem (Cat# 648463)
3. Wash buffer (PBS /2% FBS)
4. 10% formaldehyde (Polysciences # 04018); Make working solution of 1% and 1.5% formaldehyde in 1x PBS
5. Human CD19 Alexa Fluor 488 conjugate, Invitrogen (Cat# MHCD1920)
6. Human CD3 PE-Texas Red conjugate, Invitrogen (Cat# MHCD0317)
7. Biotin – xx phalloidin, Invitrogen (Cat# B7474). Phalloidin solution is 10µL Biotin – xx phalloidin in 100µL PermWash buffer.
8. Streptavidin – Alexa Fluor 647 conjugate, Invitrogen (Cat# S32357) Working solution1µL Streptavidin – Alexa Fluor 647 in 100µL PermWash buffer.
9. 10x DAPI: 10µg/mL DAPI (dissolved in dH2O, Molecular Probes Cat# D3571) and 1% Triton X-100 (from 10% Calbiochem Cat # 648463) in 1x PBS
10. 1.5mL Siliconized polypropylene microcentrifuge tubes: Sigma (Cat. T4816)

Cell preparation

Human T-cells were purified from whole blood using ResettSep Human T cell Enrichment cocktail (cat# 15021). Raji B cells were incubated for 15 minutes with 5ug/mL of Staphylococcal enterotoxin B (SEB) to make APCs. The T-cells were incubated with the Raji–APCs for 45 min at 37°C.

Staining Protocol

All washes done at 200 x g 4min 4°C in a swinging bucket rotor. Staining should be done in the dark at RT. Cell concentration should be 2 x10^6 cells per 100µL.

1. Treat cells to induce T-cell-APC conjugates, cells should be in ~500µL media.
2. Fix cells in 1.5mL of 1.5% formaldehyde for 30 minutes.
3. Wash with wash buffer, spin and resuspend in 100µL of the wash buffer.
4. Add CD3 and CD19 antibodies incubate for 30 min.
5. Wash with PermWash Buffer, spin and resuspend in 100µL of Phalloidin solution in PermWash buffer for 30 min.

Immune Synapse Staining Protocol

6. Wash with PermWash buffer, spin and resuspend in 100µL of the secondary AF 647 in PermWash buffer solution for 30 minutes.
7. Wash with wash buffer, spin and resuspend in 100µL 1% formaldehyde.
8. Add 10x DAPI solution so the concentration is 1µg/mL.
9. Run directly on ImageStream or FlowSight in 1.5 mL microcentrifuge tubes.