Simultaneous measurement of cell surface immunofluorescence, viability, and DNA content

Materials

Buffer (1 X PBS without Ca++ and Mg++, 2% newborn calf serum (NCS),

0.1% sodium azide (NaAz)

Nucleic acid staining solution (NASS, phosphate-citrate buffer tablets, sodium chloride, sodium ethylene-diaminetetraacetic acid (EDTA), bovine serum albumin (BSA), all from Sigma-Aldrich), see recipe

Ribonuclease A (RNAse) (e.g., Cat# R4875 Sigma-Aldrich, St. Louis, MO)

Dimethylsulfoxide (DMSO)

Saponin (e.g., powder from Sigma-Aldrich, or 1% solution from eBioscience, San Diego, CA)

7-amino-actinomycin D (7-AAD, e.g, Cat #129935, EMD Millipore, MA), stock solution, see recipe

TO-PRO-3 iodide (TP3) (e.g., from LifeTechnologies, Grand Island, NY)

Actinomycin D (AD, Sigma-Aldrich) stock solution, see recipe

Method

1. Place 1 x 10^6 PBS-washed cells into a 12 x 75 mm tube and add 250 µl of buffer containing 4 µg/mL of 7-AAD. Mix well.
2. For staining of cell surface antigens, add appropriate amounts of monoclonal antibodies (mAb) conjugated to fluorochromes compatible with the emissions of 7-AAD and TP3 or labeled isotypic control antibodies. Incubate the samples while protected from light for 15 min at 20°C - 25°C.
3. Wash cells once with 2 mL of 1 X PBS by centrifugation at 250 x g for 5 min. Remove the supernatant completely. Add 2 mL of 1 X PBS containing 4 µg/ml of AD. Vortex immediately, then spin cells down for at least 5 min at 250 x g, and remove the supernatant completely.
4. Resuspend cells in 0.5 mL of NASS containing 0.02% of saponin, 4 µg/mL of AD, 0.5 µM of TP3, and 200 µg/mL of RNAse followed by incubation for 30 min at 20°C - 25°C.
5. If samples were cell surface stained with mAbs other than FITC, they are acquired on the flow cytometer in their staining solution after the last incubation.
6. If samples were cell surface labeled with a FITC-conjugated mAb alone or with such a mAb and mAbs conjugated to other fluorochromes, spin cells down after DNA staining by centrifugation at 250 x g for 5 min; then, resuspend the cell pellet in 0.5 mL of 1 X PBS at pH 7.2 containing 0.02% of saponin, 0.5 µM of TP3, and 4 µg/ml of AD to restore the FITC fluorescence that is markedly diminished at pH 4.8. Then, acquire samples on the flow cytometer in the staining solution.

   Note: We have successfully used FITC, phycoerythrin (PE), and AlexaFluor488 for cell surface antigen staining, however, other fluorochromes may be negatively affected by the low pH of the NASS.

Preparation of solutions:

7-AAD stock solution (1mg/ml): dissolve 1 mg of 7-AAD powder first in 50 µL of DMSO, then add 950 µL of 1 X PBS; keep at 4°C protected from light.

Nucleic acid staining solution (NASS, pH 4.8): 0.15 M NaCl in 0.1 M phosphate-citrate buffer containing 5 mM sodium EDTA and 0.5% BSA fraction V.

Dissolve 2 tablets of phosphate-citrate buffer in 100 mL of distilled H_2O to make a 0.1 M solution.

Add 0.18 g of disodium EDTA to a final concentration of 5 mM.

Add 0.9 g of NaCl to a final concentration of 0.15 M.

Add 0.5 g of BSA to a final concentration of 0.5%.

Keep at 4°C.

Actinomycin D (AD) stock solution (1mg/ml): dissolve 1 mg of AD powder first in 50 µl of DMSO, then add 950 µl of 1 X PBS, keep at 4^{o}C protected from light.

Reference:

Schmid I, Hausner MA, Cole SW, Uittenbogaart CH, Giorgi JV, Jamieson BD. Simultaneous flow cytometric measurement of viability and lymphocyte subset proliferation. *J Immunol Meth*. 247:175-186, 2001.