Simultaneous measurement of cell surface immunofluorescence and DNA/RNA content

Materials

Phosphate buffered saline (1 X PBS without Ca++ and Mg++)

Newborn calf serum (NCS)

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, St. Louis, MO), see recipe

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

Pyronin Y(G) (PY) (e.g, from Sigma-Aldrich or AAT Bioquest, Sunnyvale, CA)

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

Method

Place 1 x 10^6 PBS-washed cells into a 12 x 75 mm tube. Add 100 µl of PBS supplemented with 2% NCS and 0.1% NaAz and mix well.

For staining of cell surface antigen expression add appropriate amounts of labeled monoclonal antibodies (mAb) or of corresponding labeled isotypic control antibody and incubate the samples while protected from light for 15 min at 20ºC - 25ºC. Note: Make sure that the fluorochromes selected are compatible with the emissions from PY and 7-AAD, respectively. FITC fluorescence is compatible, however, it is greatly diminished at the low pH of the NASS. We have used AlexaFluor488 and APC successfully, however, other fluorochromes may be negatively affected by the low pH of the NASS.

Wash cells once with 2 mL of 1 X PBS by centrifugation at 250 x g for 5 min.

Resuspend cells in 0.5 mL of NASS containing 0.02% of saponin and 10 µg/mL of 7-AAD followed by incubation for 20 min at 20ºC - 25ºC protected from light.

Then, add 1 mL of 1 X PBS and spin cells down by centrifugation at 250 x g for 5 min. Resuspend the cell pellet in 0.5 mL of NASS containing 0.02% of saponin and 10 µg/mL of AD and place the mixture on ice protected from light for 5 min.

Add 0.5 µL of a 1mg/mL stock solution of PY in distilled water. Vortex immediately and keep on ice protected from light for at least 10 min before sample acquisition on the flow cytometer.

Note: it is possible to keep cells for a maximum of three days protected from light at 4ºC in the staining solution without adverse effects.

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 4ºC 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 H20 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 4ºC.

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 4ºC protected from light.

Reference: Schmid I, Cole SW, Korin YD, Zack JA, Giorgi JV. Detection of cell cycle subcompartments by flow cytometric estimation of DNA-RNA content in combination with dual-color immunofluorescence. Cytometry 39:108-116, 2000.