

## DNA / RNA Quantitation using 7AAD and Pyronin Y

### 1. Test principle

Cell cycle analysis can be done by means of differential staining of DNA and RNA. Determining the RNA content in addition to DNA allows to discriminate  $G_0$  from different stages within  $G_1$  cells. Nucleic acid staining was performed at low pH in the presence of saponin. DNA was stained with 7-aminoactinomycin D (7-AAD) and RNA with pyronin Y(G) (PY)

### 2. Specimen

Cells of interest in suspension

### 3. Materials and reagents

- 12 x 75 mm polypropylene tubes
- Phosphate buffered saline (1 X PBS without  $Ca^{++}$  and  $Mg^{++}$ )
- Fetal Bovine serum (FBS)
- Sodium azide (NaAz)
- 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
  - i. Dissolve 2 tablets of phosphate-citrate buffer in 100 ml of distilled  $H_2O$  to make a 0.1 M solution.
  - ii. Add 0.18 g of disodium EDTA to a final concentration of 5 mM.
  - iii. Add 0.9 g of NaCl to a final concentration of 0.15 M.
  - iv. Add 0.5 g of BSA to a final concentration of 0.5%. Keep at  $4^{\circ}C$ .
- Dimethylsulfoxide (DMSO)
- Saponin (Sigma)
- 7-amino-actinomycin D (7-AAD) stock solution (1mg/ml): dissolve 1 mg of 7-AAD powder first in 50  $\mu$ l of DMSO, then add 950  $\mu$ l of 1 X PBS; keep at  $4^{\circ}C$  protected from light.
- Pyronin Y(G) (PY) (Polysciences, Inc.) : Dissolve 1 mg of PY in 1 mL of  $H_2O$ . Store at  $4^{\circ}C$  protected from light.
- Actinomycin D (C1) (AD, Roche Molecular Biosystems) stock solution (1mg/ml): dissolve 1 mg of AD powder first in 50  $\mu$ l of DMSO, then add 950  $\mu$ l of 1 X PBS, keep at  $4^{\circ}C$  protected from light.

### 4. Controls

- Unstained sample
- Pyronin Y stained single color sample
- 7AAD stained single color sample

### 5. Procedure

1. Prepare  $1 \times 10^6$  PBS- washed cells into 12 x 75 mm tubes.
  2. **Stain cells with 7 AAD:**
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- a. Resuspend PBS-washed cells in 0.5 ml of Nucleic acid Staining Solution(NASS) containing 0.02 % saponin and 10 µg/mL of 7AAD.
- b. Incubate for 20 min at 20°C- 25°C (room temperature), protected from light.
3. Add 1 mL of 1x PBS to each sample. Collect the cells by centrifugation at 250 xg for 5 min. Discard the PBS.
- 4. Stain cells with Pyronin Y:**
  - a. Resuspend each cell pellet in 0.5 mL of NASS containing 10 µg/mL of AD. Place the mixture on ice for 5 min, protected from light.
  - b. Add 5 µL of a 1:10 dilution of the PY stock solution (made with distilled water), and vortex immediately. Keep the cells on ice, protected from light, for at least 10 min before sample acquisition on the flow cytometer.
  - c. On occasion, cells can be kept for a maximum of 3 days protected from light at 4°C in the staining solution before sample acquisition on the flow cytometer.
5. Run samples on the flow cytometer.

**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.

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