DNA / RNA Quantitation using Pyronin Y and Hoechst 33342

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. Pyronin Y interacts with double-stranded RNA and double-straned DNA by intercalation. Since interactions of PY with DNA are suppressed in the presence of the DNA fluorochrome Hoechst 33342, PY can be used as the RNA-specific fluorochrome.

2. Specimen

Cells of interest in suspension

3. Materials and reagents

- 12 x 75 mm polypropylene tubes
- PBS, ice cold
- 70 % ethanol, ice cold
- Hanks' balanced salt solution (HBSS) containing Mg^{2+} and Ca^{2+}, ice cold
- Pyronin Y (PY) – Hoechst 33342 staining solution , ice cold
    - 2 mg Hoechst 33342 (mol. wt. 652, Molecular Probes)
    - 4 mg Pyronin Y (Polysciences)
    - Add HBSS containing Mg^{2+} and Ca^{2+} to 1 liter
    - Prepare fresh
- Centrifuge, 4 C
- Flow cytometer equipped with a UV laser and a 488 nm laser

4. Controls

- Unstained cells
- PY-Hoechst 33342 stained cells – G0 arrested cells
- PY-Hoechst 33342 stained cells – untreated control cells

5. Procedure

1. Prepare 1x 10^6 cells ice-cold PBS- washed cells (at 1x 10^6 cells/ml) into 12 x 75 mm tubes.
2. **Fixation with 70% ethanol:**
    a. With a Pasteur pipet transfer 1 ml cell suspension to a 15 ml glass tube containing 10ml ice-cold 70% ethanol. Fix cells for more than 2 hr.

To minimize cell clumping, rapidly injecting the cell suspension into the fixative, rather than layering onto the surface and then mixing, is preferable. The reverse order (i.e., addition of ehtanol to cellsuspensions) results in more extensive cell loss due to cell aggregation and adherence of cells ot the glass surface. The time of fixation in ethanol may vary from 2 hr to several months at 4 C.

b. Cenctriguge tubes 5 min at 300 x g, 4 C. Remove all ethanol, rinse cells once with ice-cold HBSS containing Mg^{2+} and Ca^{2+}, and suspend in ice-cold HBSS containing Mg^{2+} and Ca^{2+} and, at a density at 1 x 10^6 cells/ml.

3. Stain cells with Pyronin Y and Hoechst 33342:

a. Mix 0.5 ml cell suspension with 0.5 ml ice-cold PY-Hoechst 33342 staining solution in a small tube. Keep sample in the dark.

4. Measure cell fluorescence 20 min after addition of the PY-Hoechst 33342 staining solution.

Reference: Current Protocols in Cytometry (1997), Contributed by Zbigniew Darzynkiewicz and Gloria Juan