

DNA Staining with Propidium Iodide for Cell Cycle Analysis

Solutions to Prepare:

1. RNase A solution: 2mg/mL in HBSS.
2. Propidium Iodide: 0.1 mg/mL (Sigma) in HBSS with 0.6% NP-40 (300 μ L in 500mL)
3. 1X HBSS with 2% FBS (10mL FBS in 500 mL HANKS)
4. 70% Ethanol (keep on ice)

Method:

1. Prepare cell suspensions in the normal manner for the cell line/type of interest.
2. Adjust concentrations to 2×10^6 cells/mL.
 - a. Centrifuge cells for 5 min at 1000 rpm
 - b. Aspirate supernatant using a vacuum
3. Suspend cells in 50 μ L of HBSS containing 2% FBS (pipette up and down a few times if your cells readily form aggregates).
 - a. Add 1mL of ICE cold 70% ethanol in a dropwise manner while mixing gently on a vortex (minimizes new aggregate formation)
 - b. Store on ice for 30 minutes to one hour
 - c. This is a good place to stop if you intend to stain the cells at another time. Cells can be stored in ethanol for several weeks to months.
4. Pellet cells and carefully remove supernatant (fixed cells will not sediment as tightly as live cells, take care not to aspirate to dryness and lose the pellet).
 - a. Wash 2X with HBSS + 2% FBS
5. Add 500 μ L of PI solution to pellets, vortex gently
6. Add 500 μ L of RNase Solution to tubes and vortex
7. Incubate in the dark for 30 minutes at RT
8. Filter through 40 μ M mesh filter
 - a. Keep cold and in the dark until ready to analyze.

NOTES on Cell Preparation:

Counting is the most important step.

Proper DNA saturation is the key to good cell cycle analysis and should not vary between samples. The variance comes from too much/too little DNA per amount of PI added to the sample resulting in shifting DNA content peaks i.e. G1 will not appear at the same place on the scale for each sample. This applies to all stoichiometric DNA dyes.

To learn more about cell cycle modeling and analysis of DNA Content:

1. Shackney, S.E. and Shankey, T.V. 1999 **Cell Cycle Models for Molecular Biology and Molecular Oncology: Exploring New Dimensions** *Cytometry* 35:2 p. 97-116
2. Shankey, T.V., Rabinovitch, P.S., Bagwell, B., Bauer, K.D., Duque, R.E., Hedley, D.W., Mayall, B.H., and Wheelless, L. 1993 **Guidelines for Implementation of Clinical DNA Cytometry** *Cytometry* 14: p.472-477
3. Consensus on DNA analysis by flow cytometry (Book). M.G. Ormerod, B. Tribukait, Walter Giaretti.
4. Darzynkiewicz, Z. and Juan, G. 2001. DNA Content Measurement for DNA Ploidy and Cell Cycle Analysis. *Current Protocols in Cytometry*. 00:7.5:7.5.1–7.5.24.

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