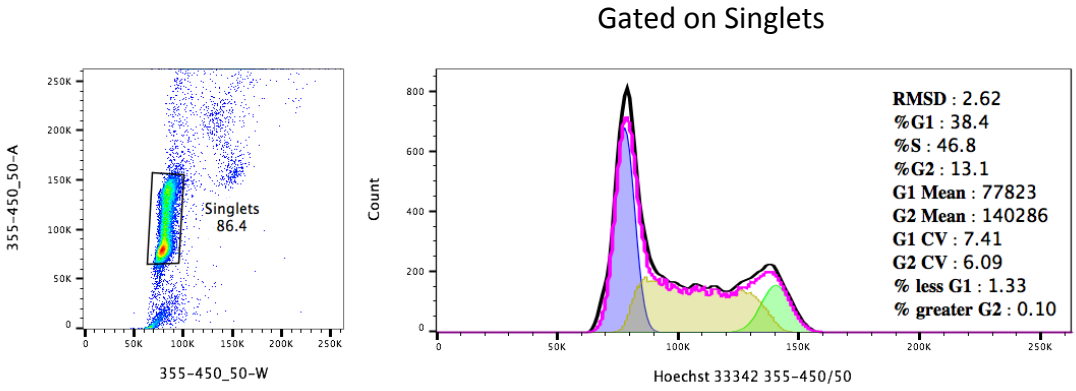
Hoechst 33342 Staining for Cell Cycle Analysis of Live Cells

Specificity: Binds preferentially A-T base regions in DNA

The optimal Hoechst 33342 dye concentration and staining time may vary between different cell types, as dye uptake depends on cellular metabolic rates; therefore, both have to be determine empirically. In general, dye concentrations between 1 and 10µg/ml, and incubation times between 20 and 90 min, will produce DNA histograms with acceptable coefficients of variation. Because Hoechst DNA staining is performed on unfixed cells, it is possible to use other nonvital DNA dye (PI, 7AAD), for concurrent dead cell discrimination (1).



Data from William Mueller and Sandra Clauder-Münster/ Steinmetz Group

Protocol:

1. Add HO to the culture medium of the cells at 1 – 5µg/ml.
2. Incubate the cells in HO at 37°C for 30-60 minutes
3. Take out the cells from the plate/flask and analyse them without washing the media containing HO.

* Adherent cells: Perform the staining *in vitro*, trypsin and trypsin-neutralizing solutions should contain the same HO concentration.
* Improved resolution (CVs) can be obtained by adding 0.1-0.3µg/ml of DIOC5 at the same time as HO incubation.

Hoechst 33342 staining solution (HO): Prepare a stock 1mg/ml in distilled water. Do not use PBS, dye will precipitate. The HO stock is

stable for at least one month at 4°C. For long storage times keep it at -20°C.

References

1. Current Protocols in Cytometry (1997) 7.5.1-7.5.24 Copyright © 1997 by John Wiley & Sons, Inc. Contributed by Zbigniew Darzynkiewicz and Gloria Juan.