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## HOECHST 33342 DNA STAINING FOR DNA CONTENT BY FLOW CYTOMETRY (FCM)

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Hoechst 33342 (H342) DNA staining is a “vital” stain with the main advantage of avoiding cells permeabilization for labelling but do require physiologic conditions since the dye internalization is an active transport process. H342 binds preferentially to A-T base-pairs (DNA specific) and the procedure require no RNA digestion in contrast of classical DNA staining (i.e. Propidium Iodide). H342 detection require UV laser for excitation (excitation/emission spectra 343/483 nm).

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### METHOD A

1. Add 10  $\mu\text{L}$  of H342 working solution (100X) per each mL of pre warmed complete culture media to the culture bottles or plates (final concentration 5  $\mu\text{g}/\text{mL} \equiv 8 \mu\text{M}$ ).  
*Note: If cells are not adherent resuspend them with complete medium at the same H342 final concentration indicated above)*
2. Incubate 60 minutes at standard culture conditions (37°C, CO<sub>2</sub> 5%).
3. Trypsinize cells, wash them twice with PBS and resuspend them with 1 mL complete medium plus the same amount of H342 (see above) or PBS+FCS 2-5% (optimal cell concentration 1x10<sup>6</sup> cells/mL) in a FCM tube.
4. Analyze by flow cytometry immediately after incubation.

*\* Note: Do not wash H342 (must be always present on prepared samples).*

### METHOD B

1. Trypsinize cells, wash them twice with PBS and resuspend them with 1 mL complete medium or PBS+FCS 2-5% (optimal cell concentration 1x10<sup>6</sup> cells/mL) in a FCM tube.
2. Add 10  $\mu\text{L}$  of H342 working solution (100X) (final concentration 5  $\mu\text{g}/\text{mL} \equiv 8 \mu\text{M}$ ).
3. Incubate 60 minutes in a 37°C pre-warmed bath, light protected.
4. Analyse by flow cytometry immediately after incubation.

*\* Note: Do not wash H342 (must be always present on prepared samples).*

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### Reagents

- Hoechst 33342: Trihydrochloride, trihydrate  
Molecular Probes (H-1399)  
Molecular weight 615.99
- Hoechst 33342 stock solution (2000X)  
Hoechst 33342 10 mg/mL with H<sub>2</sub>O-bd  
(Store at -20°C).
- Hoechst 33342 working solution (100X)  
(1/20) stock solution with H<sub>2</sub>O-bd.  
(1 month stable at 4°C).
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