PROPIDIUM IODIDE DNA STAINING
FOR DNA CONTENT BY FLOW CYTOMETRY (FCM)

Propidium Iodide (PI) DNA staining requires cell permeabilization prior staining for dye internalization. Since PI intercalates to DNA and RNA, RNAse is used to digest RNA in order to ensure RNA staining is completely avoided.

Fixing cells:

1. Tripsinize cells (if required) and wash twice in PBS.
2. Resuspend 1x10e6 cells in PBS (0.3 mL)
3. Add 0.7 mL ice-cold (or -20°C) absolute EtOH slowly while mixing by vortex.

   (Vortex during EtOH addition is crucial to avoid aggregates. The aggregates will be stable in the fixation). (Samples can be stored in EtOH several weeks at 4°C)

4. Maintain EtOH fixation at least 2 hours on ice.

Propidium Iodide staining:

5. Pellet at 5000 rpm (or high) in a Eppenforf centrifuge.
6. Manually aspirate supernatant up to pellet level.

   (The high pellet spreading indicates pellet fragility and must handle with care. Otherwise you lose the cells).

7. Pellet twice with PBS.
8. Resuspend pellet with working solution and incubate 30 minutes at RT or preferable at 4°C overnight.

   (The 4°C overnight incubation can be maintained for 48h or longer. You must check your cells for degradation)

9. Analyze by flow cytometry

Reagents:

Propidium Iodide:	Molecular Probes (P-1304) Molecular weight 668.4
RNAse A:	DNAse free. *(If is not DNAse free, inactivate 5 minutes by temperature*).
PBS-EDTA:	5 mM EDTA in PBS.

Solutions:

A Solution:	Sodium citrate (38 mM) Propidium Iodide (500 µg/mL in water) *(Water solution stable 1 year at 4°C)*
B Solution:	RNAse A (10 mg/mL in PBS) *(PBS solution stable at -20°C)*
Working solution:	Each mL of working solution contains: 940 µL of PBS 30 µL of solution A 30 µL of solution B *(Prepare this solution daily, prior to use)*