2-Dimensional flow cytometry

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Materials Needed

- Propidium Iodide 1mg/ml
- RNase A 1mg/ml
- EdU and Click-iT reaction materials

Procedure

Cell harvest

1. Pulse the cells with media containing 10uM of EdU for 15-30 minutes in CO_2 incubator.
2. Harvest cells 3 x 10^6 in media (scrape 6cm plate or cell suspension) and transfer to 1.5 ml tube.
3. Centrifuge cells in a 1.5 ml tube for 5 min, 1000 rpm, RT.
4. Aspirate supernatant completely with P200 (will be effective if spun down ~3 sec once again to discard residual supernatant – same for all aspiration procedures below)
5. Aspirate the media and resuspend the cells with 1ml of PBS. Centrifuge.
6. Aspirate and resuspend the cells in 100μl PBS+ 1%NGS.

Cell fixation

1. Take chilled 100% EtOH to cold room.
2. place the centrifuge tube on the vortex and add 900μl of 100% EtOH dropwise to tube.
3. Mix well and keep the tube at 4°C overnight.

Click-iT reaction

1. Centrifuge 5 min, 1000 rpm, RT, then aspirate supernatant completely.
2. Add 0.5 ml of 1% BSA/PBS and incubate for 30 minutes at RT (blocking)
3. Add 1ml of 0.5% Triton X-100/PBS, resuspend by tapping.
4. Incubate 30 min, RT (dark)
5. Meanwhile prepare click chemistry cocktail <15 minutes before use.
    - Mix the ingredients in the **following order**.

Component	For 1000μl
1X Click-iT reaction Buffer	860μl
$CuSO_4$	40μl
Alexa-488 azide (1mM)	4μl
Reaction buffer additive (1X)	100μl

6. Aspirate and resuspend the cells in 500μl of the cocktail and incubate for 30 minutes at RT in dark.
7. Wash twice with 1ml of PBS.

PI staining

1. Discard supernatant and resuspend cell pellet in 1ml PBS containing 10 μg/ml RNase A and 20 μg/ml PI stock solution, transfer to FACS tubes and incubate at room temperature (RT) in the dark for 30 min. (For "EdU-only", just add PBS).
2. Transfer to a 5 ml Falcon 2054 FACS tube for FACS/LSRII analysis.
3. Adjust concentration to 2 x 106 /ml by adding 5 μg/ml PI in PBS.