ImageStream® Mitotic Index and Cell Cycle analysis of Jurkat Cells

Experimental Procedures:

Samples (4x10⁶ cells per test):

Unstained and single fluorescent color control samples

Control	Cell type
Unstained	Jurkat
Tubulin AF 488	Spectral compensation of Alexa Fluor 488
DRAQ-5	Spectral compensation of Draq-5
Tubulin/Dq5	Jurkat

Stain according to following protocol.

Materials

01. Anti Tubulin biotin : Molecular Probes / Invitrogen (Cat# A-21371) [0.5ug/ul]
02. Streptaviding Alexa Fluor 488 : Molecular Probes / Invitrogen (Cat# S-32354) [2ug/ul]
03. DRAQ5: Biostatus/Alexis SKU:DR50050, 5 mM stock
04. Cytofix / Cytoperm : BD (Cat# 51-2090k2)
05. Jurkat cells, clone E6-1: ATCC (TIB-152)
06. RPMI : Gibco (Cat# 21870) with recommended supplements
07. PBS : Gibco (Cat# 14190)
08. Paraformaldehyde "PFA": E.M.S. (Cat# 15713) [20% in diH_2O]

Optional Materials

01. 7AAD: Molecular probes / Invitrogen (Cat# A-1310) [1mg/ml]. Ch.5 nuclear image.
02. Rnase-A: Sigma (Cat#R-4642) [27.5mg/ml or 90KU]. Use 1:100.

Cell preparation

We used Jurkat cells cultured in RPMI supplemented with 5% fetal calf serum, nonessential amino acids, sodium pyruvate and penstrep L-glutamine, in an incubator containing 5% CO_2 at 37° C. All washes were performed at 300 x g for 10' at RT. All stains done at $4x10^7$ cells/ml on ice.

Anti Tubulin Cell Cycle

Procedure

01. Split cells in T-75 to 3x10^5th cells per ml in RPMI buffer with 10%FBS.
02. Allow cells to reach log phase growth over night and harvest the next morning.
03. Wash cells 1x with 5ml PBS 2% FBS.
04. Resuspend pellet in 300ul BD cytofix/cytoperm buffer.
05. Incubate 20min at 4°C (as the BD protocol recommends).
06. Dilute BD perm wash buffer 1:10 with diH_2O and use for subsequent perm wash steps.
07. Wash 2x in BD perm wash buffer.
08. Resuspend pellet in 100ul of BD perm wash buffer with 1:1000 dilution [0.05ug/ul] of the anti-tubulin biotin antibody.
09. Incubate on ice 30min.
10. Wash cells 1x 500ul perm wash buffer.
11. Resuspend pellet in 100ul perm wash buffer with the strepavidin AF-488 at 1:1000 [0.02ug/ul]. Titrate AF-488 dilution to optimize brightness for each fix and cell type.
12. Incubate on ice 30min.
13. Wash cells 2x in 500ul perm wash buffer.
14. Resuspend cells in 100ul PBS 1%PFA running buffer.
15. Add 0.5ul of Draq-5. Titrate Draq-5 dilution to optimize brightness for each fix and cell type.
16. Run on ImageStream.