Creighton UNIVERSITY

Cytoplasmic c-myc Expression by Flow Cytometry

(Greg A. Perry, Ph.D.)

Reagents:

Perm-Wash buffer
Fix-Perm reagent
FACSFix

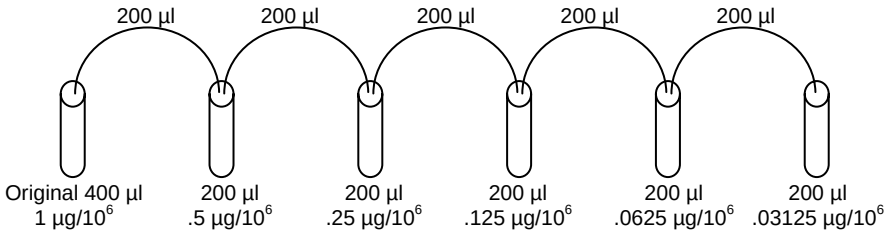
Method:

I. Prepare Cells

- Harvest cells (vector positive (v^+) and vector negative (v^-) 293 cells by gently washing off of dish.
- Centrifuge cells 10 minutes @ 350g (4ºC).
- Remove supernatant and resuspend cells in 2ml PBS4.
- Adjust 293 cells to $1x10^6$ cells/ml in PBS4.
- Place 1 ml of the cells into 14 separate 12x75 mm tubes
  8 tubes for each cell type (labeled 1-8 for v^+ and 9-16 for v^-)

II. Prepare Antibody

- Place 4 µg (3.64 µl) of antibody in an eppendorf tube.
  - Using anti-RAG #307
  - 1.1 mg/ml (=1,100 µg/ml)
  - in 25mM Tris buffer (pH 7.6) with 150mM NaCl & 3mM KCl
- Add 20 µl of the Zenon labeling reagent (Kit component A)
- Incubate at room temperature in the dark for 5 minutes.
- Add 20 µl of the Zenon blocking reagent (Kit component B).
- Incubate at room temperature in the dark for 5 minutes.
- Bring up the total volume to 400 µl by adding 376.4 µl Perm/Wash buffer.
- Do doubling dilutions of the antibody in Perm/Wash buffer as follows:



III. Fix, permeablize & stain the cells

- Centrifuge cells 5 minutes @ **250g** (4ºC).
- Remove supernatant.
- Remove tubes 1 (v^+ cells only) and 9 (v^- cells only). Resuspend these two tubes in 1 ml FACSFix and place on ice.
  All of the rest of the procedures will be performed on tubes 2-8 and 10-16 only.
- Resuspend tubes 2-8 and 10-16 in 100 µl of Fix&Perm reagent and mix gently.
- Incubate 20 minutes on ice.
- Add 1 ml Perm/Wash buffer and mix gently.
- Centrifuge 5 minutes @ 250g (4ºC).
- Remove supernatant.

- Remove tubes 2 (v^+ perm. cells only) and 10 (v^- perm. cells only). Resuspend these two tubes in 1 ml FACSFix and place on ice.
  All of the rest of the procedures will be performed on tubes 3-8 and 11-16 only.
- Resuspend tubes 3-8 and 11-16 in 100 µl of antibody as follows:

1 µg/10^6	tubes 3 and 11
.5 µg/10^6	tubes 4 and 12
.25 µg/10^6	tubes 5 and 13
.125 µg/10^6	tubes 6 and 14
.0625 µg/10^6	tubes 7 and 15
.03125 µg/10^6	tubes 8 and 16

- Mix gently and incubate 30 minutes at room temperature in the dark.
- Add 1 ml Perm/Wash buffer and mix gently.
- Centrifuge 5 minutes @ 250g (4ºC).
- Remove supernatant.
- Resuspend in 1 ml Perm/Wash buffer and mix gently.
- Centrifuge 5 minutes @ 250g (4ºC).
- Remove supernatant.
- Resuspend in 1 ml Perm/Wash buffer and mix gently.
- Centrifuge 5 minutes @ 250g (4ºC).
- Remove supernatant.
- Resuspend cells in 1 ml FACSFix.
- Analyze on the cytometer.