Immunofluorescence Assay for Bax activation

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Setting up the cells and basic processing is the same as for the standard immunofluorescence protocol

Fixation

1. Add 30ul of 4% Paraformaldehyde for 30 min @ room-temperature (RT).
2. Wash 1x with PBS for 5 min.

   Note: During washing period always place 6 well dishes on orbital shaker.

Permeabilization

1. Add 30ul of 0.2 % CHAPS in PBS for 2 min @ RT
2. Wash 1x with PBS/ 0.02% Tween 20 for 5 min
3. Wash 1x with PBS/0.02% Tween 20/ 1% BSA for 5 min

Primary antibody

1. Add 30ul of 6A7 antiboby (dilution 1:100) in PBS/3% BSA
2. Incubate in a humidified chamber for 60 min @ 37 C.

Washes

1. Wash with PBS/0.02% Tween 20/1% BSA for 5 min

Second antibody

6A7 activation is best observed by double staining. We use either cytochrome c or 2G2 (human specific integral membrane protein from the inner mitochondrial membrane) or Hsp60 (lumenal mitochondrial marker - you can buy this from Stressgen). Our cytochrome c antibody was raised in sheep and should be used at a dilution of 1:750. The 2G2 antibody is a mouse monoclonal and is used at 1:100 dilution. It can be purchased from Exalpha Biologicals. Hsp60 depends on what Stressgen is selling at the moment.

Secondary antibodies

From here on in the procedure is the same as the standard protocol.