Staining Methods for Cell Death

Z. Xia 10/2/95

I. The simplest way: trypan blue

Dead cells stain blue

II. Non-fixed cells: FDA (fluorescein diacetate)-green, alive cells; P.I. (propidium iodide)-red, dead cells

35 mm plates:

1. To 2 ml medium or PBS, add 2 ul 2 mg/ml P.I.
   6 ul 5 mg/ml FDA
2. R.T. 3 min
3. Rinse 1 X PBS
4. Leave cells in PBS. Examine cells under the scope immediately.

Note:

1. If the P.I. staining is not strong enough to be picked up easily under your scope, use 2 X P.I., i.e., 4 ul 2 mg/ml in 2 ml medium
2. After staining, need to examine the staining right away otherwise the green staining gets diffused. You can leave cells at 4ºC for a few hr - overnight to slow down the diffusion (I have tried 3T3, do not know if it works for neurons)
3. Ref.: K.H. Jones & J.A. Senft (1985) *J. Histochemistry & Cytochemistry* 33: 77–79. M. Schramm et al., (1990) *PNAS* 87: 1193–1197
4. This method stains for non-fixed cells.
5. P.I.: Sigma, dissolve in PBS
   FDA: Sigma, dissolve in acetone

III. P.I. staining for fixed cells

1. Fixation:
   a. ETOH fixation-gives brighter P.I. staining
      Gently overlay over media 4X media vol. of ETOH pre-cooled to –20ºC
      R.T. 3 min
      Gently mix media & ETOH with pipet
      R.T. 5 mi

   or

   b. Paraformaldehyde fixation: (8% paraformaldehyde/4% sucrose/in PBS, pH 7.2-7.6)
      Gently overlay over media 2X media vol. of 8% paraformaldehyde/4% sucrose/in PBS, pH 7.2-7.6
      Gently tilt the plates to mix
      R.T. 15 min
2. Aspirate off media
3. Staining:
   4 ug/ul P. I./0.1% triton X-100/0.5 mg/ml RNaseA in PBS
   R.T. 5 min

Examine under the scope or mount with coverslips

Note:

1. P.I. will stain for both DNA and RNA. It is critical to include RNase A to eliminate the cytosolic RNA staining background. If use ETOH fixation, it is less critical to include RNaseA in staining soln.
2. This will stain both alive and dead cells. Alive cells should have evenly stained nuclei. Nuclei from apoptotic cells show condensed, or fragmented morphology. Cannot distinguish necrosis.

IV. Hoescht staining

1. Fix cells
   a. Remove media, fix w/ 4% paraformaldehyde/4% sucrose in PBS, neutral pH, R.T. 15–45 min
   b. If cells are not adhering well to the plates:
      Gently overlay over media 2X media vol. of 8% paraformaldehyde/4% sucrose/in PBS, pH 7.2-7.6
      Gently tilt the plates to mix
      R.T. 15 min
2. Wash 1X PBS/0.1% triton X-100, R.T. 5 min
3. Stain cells w/2.5 ug/ul Hoeschst 33258 in PBS/0.1% triton X-100 R.T. 5 min
4. Wash 1X PBS/0.1% triton X-100, R.T. 5 min
5. Mount w/coverslips. Examine cells under fluorescence scope using 2 DAPI filter

Note:

1. Alive cells should have evenly stained nuclei. Nuclei from apoptotic cells show condensed, or fragmented morphology.
2. Hoeschst 33258, Sigma B-2883 (bis-Benzimide), 5 mg/ml in H^2O stock. Light sensitive.
3. Hoeschst 33258 stains permeablized cells; Hoeschst 33342 is permable, can stain both fixed and non-fixed cells.

To distinguish alive vs. necrotic, apoptotic cells:

Morphologically:

Alive cells: Phase bright
Necrotic: cell swelling, i.e., enlarged cell bodies, cell membrane leakage, lysis of cell body
Apoptotic: Rough membrane, plasma membrane shrinkage, cell body shrinkage, membrane blebbing, no lysis of cell body

Staining:

1. Trypan blue: dead cells stain blue. Can not distinguish necrotic vs. terminally apoptotic cells
2. FDA/P.I. staining: Alive cells stain blue, necrotic or terminally apoptotic cells stain red. Early apoptotic cells should not stain red.
3. P.I. or Hoeschst staining of fixed cells: Nuclei from apoptotic cells show condensed, or fragmented morphology.

4. Tunnel staining: Commercial kits available. Nuclei from apoptotic cells show condensed, or fragmented morphology.
5. DNA ladder: Necrotic cells do not show DNA laddering; most, but not all, of the apoptotic cells show DNA laddering.

Positive control for apoptosis:

1 uM staurosporin in media, 3-24 hr for most of the cells, always induces apoptosis (as far as we know).

Staurosporin: 1 mM stock in DMSO, 4°C