CELL DEATH DETECTION
Roche Cell Death Detection ELISA Plus

This procedure is used to measure DNA fragmentation and histone release from the nucleus during the apoptosis process.

Materials/Reagents:
From kit:
anti-histone-biotin
anti-DNA-POD
positive control
incubation buffer
lysis buffer
substrate buffer
ABTS substrate tablet
Microplate
Adhesive foil covers
Other:
Standard tissue culture reagents
Apoptotic test and control drugs
Microplate reader (visible wavelength)
Multi-pipetteman

Procedure:

Cell treatment
Plate cells the day before at an appropriate density into 96 well tissue culture plates. I diluted the cells to $1X10^5$ cells per mL, and then plated 100 cells per well for a total of $1X10^4$ cells per well.

Treat cells with desired apoptotic drug for desired time. Perform dose response and timepoints if trying drug/cell type for the first time. I used 5 μM.for Taxol , 50 μM for etoposide, and 200 ng/mL for TNFα. Perform all samples at least in duplicate. Include negative and positive controls.

Suggested treatment timepoints: 5-24 H. Cells should have initiated apoptosis but should not yet be lysed.

ELISA processing

1. Reconstitute lyophilized components.
   anti-histone-biotin in 450 μl of ddH20

anti-DNA-POD in 450 μl of ddH20
positive control in 450 μl of ddH20

2. Make ABTS substrate.
   Dependent on the number of samples (need 100 ml per sample), dissolve 1,2,or 3 ABTS tablets in 5,10, or 15 mL substrate buffer.

3. Prepare immunoreagent.
   (Need 80 μl per sample. Must be made shortly before use. Do not store.)

   For 10 samples:

Anti-histone-biotin	40 μl
Anti-DNA-POD	40 μl
Incubation buffer	720 μl
Total immunoreagent	800 μl

4. Centrifuge the 96 well plate for 10 min at 200x*g*.
   Remove supernatant and discard when looking only at apoptosis
   (keep the supernatant and analyze separately if you want to analyze necrosis as well).
5. Add 200 μl of lysis buffer per well using multipipetman.
6. Incubate 30 min at room temp.
7. Centrifuge the lysate at 200x*g* for 10 min.
8. Transfer 20μl from the supernatant into the streptavidin-coated microplate
9. Add 80 μl of immunoreagent to each well.
10. Cover the microplate with an adhesive foil. Incubate on a shaker under gentle shaking for 2 hour at room temp.
11. Remove the solution thoroughly by tapping.
12. Rinse each well 3X with 250-300 μl incubation buffer and remove solution by tapping.
13. Add 100 μl ABTS solution to each well.
14. Incubate on plate shaker for 10-20 min or until color is sufficient for photometric analysis.
15. Measure at 405 nm against ABTS solution as a blank.

Comments:
The cell density, drug concentrations, and treatment times all must be optimized.

References:
1. “Cell Death Detection ELISA Plus” pdf instruction manual available on Roche website.

2. Wang, Guttridge, Mayo, Baldwin. NF-kappa B Induces Expression of the Bcl-2 Homologue A1/Bfl-1 To Preferentially Suppress Chemotherapy-Induced Apoptosis Mol. Cell. Biol. 1999 19: 5923-5929

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