**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-pipette man

**Procedure:**

**Cell treatment**  
Plate cells the day before at an appropriate density into 96 well tissue culture plates. I diluted the cells to $1 \times 10^5$ cells per mL, and then plated 100 cells per well for a total of $1 \times 10^4$ 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 $\mu$M for Taxol, 50 $\mu$M 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 $\mu$L 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 200xg.
   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 200xg for 10 min.

8. Transfer 20 µl 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:

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