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U Michigan - Retinal Cell Death

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We use this protocol and it's working

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Abstract

Summary:

Capture nucleosomes from the cytoplasm of apoptotic cells on a micro titer plate and detect the associated histones with HRP-conjugated antibody. Designed for use with cell culture but adapted for use with homogenized mouse retinas.

Materials

MATERIALS

Cell Death Detection ELISA **Roche Catalog #**11920685001

Microcentrifuge tubes **Denville Scientific Inc. Catalog #**C2170

Note:
Roche, RRID:SCR_001326

Reagent Preparation:

Reagent 1:
Anti-Histone-Biotin (red)
Procedure: Reconstitute the lyophilizate in 4.8 ml of double distilled water for 10 minutes and mix thoroughly, aliquot and store at 2 to 8°C for up to 2 months

Reagent 2:
Anti-DNA-POD (white)
Procedure: Reconstitute the lyophilizate in 4.8 ml of double distilled water for 10 minutes and mix thoroughly, aliquot and store at 2 to 8°C for up to 2 months

Reagent 3:
Positive Control (blue)
Procedure: Reconstitute the lyophilizate in 0.5 ml of double distilled water for 10 minutes and mix thoroughly, aliquot and store at 2 to 8°C for up to 2 months

Reagent 4:
Incubation Buffer
Procedure: Make 100 ml (for one plate) by mixing 10 ml of 10X incubation buffer with 90 ml of double distilled water

Reagent 5:
Lysis Buffer
Procedure: Make 10 ml (for 32 samples) by mixing 1 ml of 10X lysis buffer with 9 ml of double distilled water

Reagent 6:
ABTS Substrate Tablet
Procedure: Dissolve 1 tablet in 5 ml of Substrate buffer and store at 2 to 8°C for up to 1 month. Protect from light. Warm up to 15 to 25°C before use.

Reagent 7:
ABTS Stop Solution

Procedure: If turbidity or precipitate is visible, warm up to 37°C with shaking until the solution is clear. Store at 2 to 8°C up to the labeled expiration date

Reagent 8:
Immuno reagent
Procedure: Mix 1 part of Anti-Histone-Biotin, 1 part of Anti-DNA-POD and 18 parts of Incubation Buffer (80 µl per test)

Troubleshooting

1 Weigh the microcentrifuge tube

2 Add the retina sample and weigh the retina with tube to obtain the retinal weight

3 Freeze the retina in liquid nitrogen and store at -80°C (or use fresh retina)

4 Add 50 µl 1x Lysis buffer to each mouse retina

5 Homogenize the retina and keep it on ice

6 Vortex and incubate the sample at room temperature for 30 minutes with gentle rocking

7 Centrifuge at 12500 rpm at 4ºC for 10 minutes

8 Collect the supernatant in fresh tube and save the pellet

9 Add 20 µl of samples, positive control (supplied in kit) and blank or negative control (lysis buffer) in each well (triplicates)

10 Add 80 µl of Immuno reagent to each well

11 Cover the plate with adhesive strip, cover with foil and incubate with gentle shacking (300 rpm) at room temperature for 2 hours

12 Carefully remove the solution by aspiration

13 Aspirate each well and wash by filling each well with 200 µl of incubation buffer 1x (bottle #4) using multi-channel pipette

14 Repeating the washing process for a total of 5 washes

15 Add 100 µl of ABTS Solution to each well and incubate on plate shaker (protect from light) until color develops (about 10 min)

16 Add 100 µl of ABTS stop solution to each well and gently tap the plate to ensure thorough mixing

17 Read the plate within 30 minutes at 405 nm using ABTS solution plus 100 µl of ATBS stop solution as blank (use reference wavelength 490 nm)

NOTE: prepare the solution shortly before use, do not store