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Working

U Michigan - Retinal Cell Death [↗](#)

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Mouse Metabolic Phenotyping Centers
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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.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=307&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Cell Death Detection ELISA	11920685001	Roche		
Microcentrifuge tubes	C2170	Denville Scientific Inc.		

MATERIALS TEXT

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)

- 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 4°C 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 shaking (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



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