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DNA damage assessment in the adult Drosophila brain via pH2Av (S137) immunostaining

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ABSTRACT

This protocol describes how to stain adult Drosophila brains with pH2Av to assess DNA damage.

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Protocol status: Working
We use this protocol and it's working

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Fly fixation and paraffin embedding

1 Fix flies of the desired genotype Overnight in 4% formalin, embedded heads in paraffin, cut 4-micron sections, and dry at 42˚C Overnight

Deparaffinization

2 Deparaffinize and bring through ethanol to water (xylene x 2, 100% ethanol x 2, 95% ethanol x 2, H2O x 2)

Antigen-retrieval

35m

3 Microwave slides in 10 mM sodium citrate for 00:15:00. Cool for 00:20:00. 35m

Stock: 100 mM sodium citrate, pH 6.0. Use at least 1 L of citrate solution in a large glass box to avoid drying

Blocking

1h 30m

4 Block in blocking solution (PBS with 0.3% Triton and 2% dry milk) for 30min to 01:00:00 1h

Antibody incubation

5 Incubate with primary antibody (Rockland pH2AV, cat # 600-401-914, dilution 1:100) overnight at room temperature

6 Wash 3 x in PBST (PBS with 0.3% Triton)

7 Incubate with fluorophore-coupled secondary antibody (Thermo Fisher) diluted 1:200 in blocking solution at room temperature

8 Wash 2 x in PBST and 1 x in PBS

Mounting

9 Mount in medium containing DAPI (Southern Biotech)