

Oct 18, 2019

Version 2

Isolation of rodent eyes for analysis of diabetic retinopathy V.2

DOI

dx.doi.org/10.17504/protocols.io.8fjhtkn



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External link: <https://www.diacomp.org/shared/document.aspx?id=46&docType=Protocol>

Protocol Citation: Timothy Kern 2019. Isolation of rodent eyes for analysis of diabetic retinopathy. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.8fjhtkn>

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Protocol status: Working

We use this protocol and it's working

Created: October 18, 2019

Last Modified: October 18, 2019

Protocol Integer ID: 28875

Keywords: Isolation of rodent eyes, diabetic retinopathy, analysis of diabetic retinopathy summary, diabetic retinopathy, diabetic retinopathy summary, rodent eye, diabetic complication

Abstract

Summary:

This protocol describes the procedure used by the DiaComp to isolate rodent eyes to phenotype for Diabetic Retinopathy.

Diabetic Complication:



Retinopathy

Troubleshooting

1 What we want:

Both eyes in formalin from at least 5 animals per experimental group. The longer the duration of diabetes, the better (C57BL/6 mice require at least 6 months diabetes before we can detect retinal microvascular lesions). Include also age-matched nondiabetic animals and diabetic controls for comparison.

2 Eye collection

All tissue needs to be collected fresh (within 10–15 min of death) to get best results.

NOTE: Tissue post-mortem more than 1 hour does not work!

- Anesthetize animal (we have not yet found that any particular anesthetic is better or worse than others).
- Place thumb and pointer finger on either side of eye. By spreading thumb and pointer finger, stretch the skin around the eye.
- Using fine, sharp scissors, make 3–5 deep (5–10mm) cuts while forcing scissors into the eye socket. The eye now should be attached only by the optic nerve leading into the brain. Push the scissor back into the eye socket again to cut the nerve, so that there is some optic nerve still attached to the eye. **Do not** push on the skull to protrude the eye and then cut horizontal across the skull; this can damage the eye. Be careful not to push or compress the eye.

See Figure Below

- Drop both eyes into 10% buffered formalin (pH 7.4) in a microfuge tube. Variances in pH seem to influence how well the tissue can be stained later, so check the pH (especially if the fixative is prepared in your lab). Label each tube with animal number. Also send a summary sheet listing all animals and experimental description with date of death; please put your name and email address on the sheet as well. Group identifications need not be on this sheet until analysis is completed. Parafilm each tube to minimize the likelihood of leaks, and put tubes in a zip-lock bag.
- We will need to receive both eyes within about 4 days of death and fixation if apoptosis is to be assessed (if apoptosis is not to be assessed, the duration in formalin is not important). Please give me a few days warning that tissue about when tissue will be shipped so that we can watch for it. Email tracking number once it is sent.

DO cut deep into the socket

DO NOT cut across the socket

