

# Organotypic explant culture of adult rat retina for *in vitro* investigations of neurodegeneration, neuroprotection and cell transplantation

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*neuroprotection, ganglion cell, organotypic culture, pharmacological screening*

## Abstract

This protocol details a method for isolating retinal tissue from adult rats as an organotypic culture to study neurobiological processes in mature tissue. It combines the efficiency and control common to *in vitro* techniques with close imitation of the *in vivo* environment. Eyes from adult rats are enucleated and the neural retina is isolated. Tissue is cut into quarters, yielding eight retinal explants per animal, and cultured at a fluid/air interface on organotypic culture membranes. Explantation can be accomplished in thirty minutes per animal. Tissue is nourished by a serum-free medium and can be viably maintained for at least two weeks *ex vivo*. Protocols are provided that describe histological processing, including techniques for whole-mount and cross-sectional immunohistochemical labelling. In addition, methods for simulating intraocular cell transplantation and pharmacological screening for neuroprotective therapies are also provided.

## Introduction

See PDF file "Explant Protocol

Introduction":[http://www.nature.com/protocolexchange/system/uploads/1738/original/Explant\\_protocol\\_Intro.pdf?1299089296](http://www.nature.com/protocolexchange/system/uploads/1738/original/Explant_protocol_Intro.pdf?1299089296)

For link to complete protocol see "Martin Lab Retinal Explant

Protocol":[http://www.nature.com/protocolexchange/system/uploads/1747/original/Martin\\_Lab\\_retinal\\_explant\\_protocol.pdf?1299090215](http://www.nature.com/protocolexchange/system/uploads/1747/original/Martin_Lab_retinal_explant_protocol.pdf?1299090215)

## Reagents

See PDF file "Explant Protocol

Reagents":[http://www.nature.com/protocolexchange/system/uploads/1739/original/Explant\\_protocol\\_Reagents.pdf?1299089329](http://www.nature.com/protocolexchange/system/uploads/1739/original/Explant_protocol_Reagents.pdf?1299089329)

## Equipment

See PDF file "Explant Protocol

Equipment":[http://www.nature.com/protocolexchange/system/uploads/1740/original/Explant\\_protocol\\_Equipment.pdf?1299089355](http://www.nature.com/protocolexchange/system/uploads/1740/original/Explant_protocol_Equipment.pdf?1299089355)

## Procedure

See PDF file "Explant Protocol

Procedure":[http://www.nature.com/protocolexchange/system/uploads/1741/original/Explant\\_Protocol\\_Procedure.pdf?1299089385](http://www.nature.com/protocolexchange/system/uploads/1741/original/Explant_Protocol_Procedure.pdf?1299089385)

Procedure.pdf?1299089460

This document includes full details for performing the following steps:

1. Prepare an organotypic adult retinal explant culture and maintain this culture. TIMING: 45-60 minutes per rat (i.e. pair of eyes) for tissue dissection; 20-30 minutes for bi-daily medium exchanges
2. Co-culture retinal explants with dissociated cell types (e.g. stem cells). TIMING: cell transplantation 15-30 minutes (add approximately 10 minutes more for each additional plate).
3. Perform the pharmacological screen of neuroprotective drugs using retinal explants. TIMING: treating explants approximately 10 minutes per plate
4. Preserve the retinal explants for histological analysis. TIMING: approximately 24 hour
5. Perform cryostat sectioning of the retinal explants and label the sections by fluorescent immunohistochemistry. TIMING: cryoprotection and embedding ~24 hours; cryostat each explant block approximately 40-60 minutes; immunohistochemistry requires 2 days.
6. Perform fluorescent immunohistochemical labelling of the whole-mount explants. TIMING: approximately 3 days

## Timing

See PDF file "Explant Protocol

Timing":[http://www.nature.com/protocolexchange/system/uploads/1743/original/Explant\\_Protocol\\_Timing.pdf?1299089510](http://www.nature.com/protocolexchange/system/uploads/1743/original/Explant_Protocol_Timing.pdf?1299089510)

## Troubleshooting

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Troubleshooting":[http://www.nature.com/protocolexchange/system/uploads/1742/original/Explant\\_Protocol\\_Troubleshooting.pdf?1299089491](http://www.nature.com/protocolexchange/system/uploads/1742/original/Explant_Protocol_Troubleshooting.pdf?1299089491)

## Anticipated Results

See PDF file "Explant Protocol Anticipated

Results":[http://www.nature.com/protocolexchange/system/uploads/1744/original/Explant\\_Protocol\\_Anticipated\\_Results.pdf?1299089550](http://www.nature.com/protocolexchange/system/uploads/1744/original/Explant_Protocol_Anticipated_Results.pdf?1299089550)

## References

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References":[http://www.nature.com/protocolexchange/system/uploads/1745/original/Explant\\_Protocol\\_References.pdf?1299089572](http://www.nature.com/protocolexchange/system/uploads/1745/original/Explant_Protocol_References.pdf?1299089572)

## Acknowledgements

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## Supplementary Files

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[Explant\\_Protocol\\_Troubleshooting.pdf](#)

[Explant\\_Protocol\\_Timing.pdf](#)

[Explant\\_Protocol\\_Anticipated\\_Results.pdf](#)

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