

## ADLER LAB TISSUE CULTURE PROTOCOLS

### **Mouse Retina Organ Cultures (Adult)**

#### 1. MATERIALS

Millicell-CM filter membrane  
Rough meshed grid support  
Corning 24 well plates

#### Cell Culture Medium (Stoppini et al., (1991) J.Neurosci. Methods 37, 173-182)

MEM with 25mM Hepes	100ml
Horse serum (heat inactivated)	50ml
Hank's Solution	50ml
Glucose	1.15g
L-Glutamine(200mM)	200µl

2. Enucleate the eye and wash in HBSS two times.
3. The following dissection should be done in HBSS. Under the microscope, remove the anterior chamber of the eye then tease away the RPE so that only neural retina remains.
4. Cut the eyecup into four wedge shaped sheets and slide the millicell membrane underneath. The ganglion cell layer should be facing up and the photoreceptors should face down.
5. Apply 30 µl of collagen type I (rat tail) so that it covers the explant and allow to gel for 10 minutes before moving.
6. Transfer the Millicell membrane into the twenty-four well dishes and add just enough growth media so that the membranes are floating but not submerged (submersion will cause cell death and gross distortion of morphology).
7. Incubate at 34°C in 5%CO<sub>2</sub>, changing media every two days

#### Collagen gel

To 30µl of rat-tail collagen type I (Collaborative biochem.), add 3 µl of 10X HBSS, pH 2.5. Next add 1.6µl of 7.5% sodium bicarbonate. Work quickly as the gel will polymerize quickly. It should turn from a yellow color to an orange/pink color.

#### ORDERING INFO

Catalog #	Company	Description	Size	Contact
	Collaborative biochem; BD biosciences	collagen type I		