

Materials List for:

# A Co-Culture Method to Study Neurite Outgrowth in Response to Dental Pulp Paracrine Signals

Courtney Barkley<sup>1</sup>, Rosa Serra<sup>1</sup>, Sarah B. Peters<sup>1</sup>

<sup>1</sup>Cell, Developmental and Integrative Biology Department, University of Alabama at Birmingham

Correspondence to: Sarah B. Peters at [peters.1026@osu.edu](mailto:peters.1026@osu.edu)

URL: <https://www.jove.com/video/60809>

DOI: [doi:10.3791/60809](https://doi.org/10.3791/60809)

## Materials

Name	Company	Catalog Number	Comments
5-Fluoro-2'-deoxyuridine	Sigma-Aldrich	F0503	Used as a mitotic Inhibitor at 15 $\mu$ M concentration in co-culture media, Day 2
24 Well Cell Culture Plate	Corning	3524	Co-culture plate
Alexa-546 anti-chicken	Invitrogen	A-11040	Secondary to stain neurite outgrowth labeled by anti-GFP antibody, 1:500 dilution
Anti-GFP Antibody	Aves Lab, Inc	GFP-1010	Primary antibody to label Thy1-YFP neurons, 1:200 dilution
Anti-Neurofilament 200 antibody	Sigma-Aldrich	NO142	Monoclonal primary antibody to label neurons, 1:1000 dilution, alternative if YFP mice are not available
B6;129- Tgfb2tm1Karl/J	The Jackson Laboratory	12603	Tgfb2 <sup>fl/fl</sup> mouse model used for dental pulp cells in optimized protocol
B6.Cg-Tg(Thy1-YFP)16Jrs/J	The Jackson Laboratory	3709	Thy1-YFP mouse model genotype used for trigeminal neurons
Collagenase Type II	Millipore	234155-100MG	Used to disperse trigeminal neurons
Fetal Bovine Serum	Gibco	10437	Additive to co-culture media
Fine forceps	Fine Science Tools	11413-11	Fine forceps for TG dissection
Laminin	Sigma-Aldrich	L2020	Coats the transwell inserts at final concentration of 10 $\mu$ g/ml, stock solution is assumed at 1.5 mg/ml
Lysis Buffer (Buffer RLT)	Qiagen	79216	Extracts RNA from dental pulp cells post co-culture
L-Glutamine	Gibco	25030081	Additive to co-culture media
Micro-dissecting scissors	Sigma-Aldrich	S3146-1EA	Dissection scissors to open skull
Microscope Cover Glass	Fisherbrand	12-545-81	Circular coverslip for optional cell culturing and immunofluorescence processing
Minimal Essential Medium a	Gibco	12571063	Co-culture media base
Penicillin-Streptomycin	Gibco	15070063	Antibiotic additive to co-culture media
Phosphatase Inhibitor	Sigma-Aldrich	04 906 837 001	Additive to RIPA Buffer for extracting protein from dental pulp cells post co-culture
Polybrene	Millipore	TR-1003-G	Used to aid in dental pulp cell transfection

Poly-D-Lysine	Sigma-Aldrich	P7280	Coverslip coating to aid dental pulp cellular adhesion
Protease Inhibitors	Millipore	05 892 791 001	Additive to RIPA Buffer for extracting protein from dental pulp cells post co-culture
RNAse/DNAse free eppendorf tubes	Denville	C-2172	Presterilized 1.7 ml tubes for RNA, DNA or protein collection at the end of assay
ThinCert Cell Culture Insert	Greiner Bio-One	662631	Transwell inserts for trigeminal neurons in co-culture assays
Trypsin-EDTA (0.25%)	Gibco	25200056	Used to disperse dental pulp cells
Trypsin Type II	Sigma-Aldrich	T-7409	Used to disperse trigeminal neurons
Ultra Fine Forceps	Fine Science Tools	11370-40	Ultra fine forceps for dissection
Uridine	Sigma-Aldrich	U3750	Used as a mitotic inhibitor at 1 $\mu$ M concentration in co-culture media, Day 2
Vacuum Filtration System	Millipore	SCNY00060	Steriflip disposable filter, 50 $\mu$ m nylon net filter
Vial forceps	Fine Science Tools	110006-15	Long forceps for tissue transfer to conicals