

Isolation, Culture, and Characterization of Primary Schwann Cells, Keratinocytes, and Fibroblasts from Human Foreskin

Mashanipalya G. Jagadeeshaprasad¹, Prem Kumar Govindappa¹, Amanda M. Nelson², John C. Elfar¹

¹Department of Orthopaedics and Rehabilitation, Center for Orthopaedic Research and Translational Science (CORTS), The Pennsylvania State University College of Medicine ²Department of Dermatology, The Pennsylvania State University College of Medicine

Corresponding Author

John C. Elfar

openelfar@gmail.com

Citation

Jagadeeshaprasad, M.G., Govindappa, P.K., Nelson, A.M., Elfar, J.C. Isolation, Culture, and Characterization of Primary Schwann Cells, Keratinocytes, and Fibroblasts from Human Foreskin. *J. Vis. Exp.* (181), e63776, doi:10.3791/63776 (2022).

Date Published

March 23, 2022

DOI

10.3791/63776

URL

jove.com/video/63776

Materials

Name	Company	Catalog Number	Comments
0.22 µM sterile filters (Millex-GP Syringe Filter Unit, polyethersulfone)	MilliporeSigma	SLGPR33RS	
70 µM cell strainers	CELLTREAT	229483	
100 µM cell strainers	CELLTREAT	229485	
1 mL disposable syringes	BD Luer-Lok	BD-309659	
5 mL disposable syringes (Syringe sterile, single use)	BD Luer-Lok	BD309646	
10 mL disposable syringes	BD Luer-Lok	BD305462	
1% TritonX-100	Sigma	X100-1L	Prepared at the time of use
4% paraformaldehyde solution	ThermoFisher Scientific	J19943.K2	Ready to use and store at 4 °C
5% BSA	Sigma	A7906-100G	Prepared at the time of use
70% ethanol	Pharmco	111000200	
Antibiotic	ScienCell Research	503	
Chemometec Vial1-Cassette	Fisher Scientific	NC1420193	
Collagenase	Gibco	17018-029	
Coverslip	Fisherbrand	12544D 22*50-1.5	
Dispase I	Sigma-Aldrich	D46693	
DMEM basal medium	ScienCell Research	9221	
Dulbecco's phosphate-buffered saline free from Ca ²⁺ and Mg ²⁺ (DPBS)	Corning	21-031-CV)	
Nunc 15 mL Conical Sterile Polypropylene Centrifuge Tubes	ThermoFisher Scientific	339651	
Nunc 50 mL Conical Sterile Polypropylene Centrifuge Tubes	ThermoFisher Scientific	339653	
1.5 mL micro-centrifuge tubes	Fisherbrand	02-681-5	
Fetal bovine serum (FBS)	ThermoFisher Scientific	10082147	

Fibroblast complete medium	ScienCell Research	2331	
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Invitrogen	A11032	Dilution (1:500)
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A11008	Dilution (1:500)
Hanks' Buffered Saline Solution (HBSS buffer)	Lonza	CC-5022	
Human foreskin			De-identified human foreskin tissue for research purposes (Institutional Review Board- IRB #17574).
KGM-GOLD keratinocyte medium (KGM gold and supplements)	Lonza	00192151 and 00192152	
Mouse alpha-smooth muscle actin antibody	ThermoFisher Scientific	14-9760-82	Dilution (1:200)
Mouse Cytokeratin14 antibody	Abcam	ab7800	Dilution (1:100)
Mouse S100 antibody	ThermoFisher Scientific	MA5-12969	Dilution (1:200)
Multi chambered (4 well glass slide)	Tab-Tek	154526	
NucleoCounter -Via1-Cassette	Chemometec	941-0012	
Poly-L-Lysine (PLL)	ScienCell Research	32503	
ProLong Gold Anti-fade Mountant with DAPI	Invitrogen	P36935	
Rabbit K10 antibody	Sigma-Aldrich	SAB4501656	Dilution (1:100)
Rabbit p75-NTR antibody	Millipore	AB1554	Dilution (1:500)
Rabbit vimentin	ProteinTech	10366-1-AP	Dilution (1:200)
Schwann cell culture medium	ScienCell Research	1701	
Precision tweezers DUMONT straight with extra fine tips Dumostar, 5	ROTH	LH75.1	Sterilize with 70% alcohol before use
IRIS Scissors, sharp/sharp. Length 4-3/8"(111mm)	Codman	54-6500	Sterilize with 70% alcohol before use
Sterilized surgical - sharp blade (Duro Edge Economy Single Edge Blades)	Razor blade company	94-0120	Sterilize with 70% alcohol before use
T25 culture flask	Corning	353109	
Trypsin neutralization buffer (TNS)	Lonza	CC-5002	
Trypsin/EDTA	Lonza	CC-5012	
Inverted microscope	ZEISS	Axio Observer 7- Axiocam 506 mono – Apotome.2 microscope	For immunofluorescence of chamber slide containing stained cells
Inverted microscope	ZEISS	Primovert	For visualizing/observing cell attachment or detachment