

# Determining the Toxicity of UV Radiation and Chemicals on Primary and Immortalized Human Corneal Epithelial Cells

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jove.com/video/62675

## Materials

Name	Company	Catalog Number	Comments
75 cm <sup>2</sup> Vented Flask	Corning	354485	This is the BioCoat brand, collagen-coated
96 well plate	costar	3370	
alamarBlue	Fisher Scientific	dal 1025	
Annexin Staining buffer solution	Invitrogen, Burlington, ON, Canada		
Annexin V	Invitrogen, Burlington, ON, Canada		
Axiovert 100 microscope with a Zeiss confocal laser scanning microscope 510 system	Carl Zeiss Inc., Germany		
Corning 48 Well plates	Corning	354505	This is the BioCoat brand, collagen-coated
Cytation 5	BioTek	CYT5MPV	Can read fluorescence from 280 - 700 nm (for assay 540/590)
Fetal Bovine Serum	Hycone	SH30396.03	
glass bottom coverslips	MatTek Corporation, Ashland, MA, USA		
Human Corneal Epithelial Cells	University of Ottawa	N/A	SV40-immortalized human corneal epithelial cells from Dr. M. Griffith (Ottawa Eye Research Institute, Ottawa, Canada) and have been characterized Griffith M, Osborne R, Munger R, Xiong X, Doillon CJ, Laycock NL, Hakim M, Song Y, Watsky MA. Functional human corneal equivalents constructed from cell lines. <i>Science</i> . 1999;286:2169-72.
Human Ocular Epithelia Media (HOEM) with the following supplements:	Millipore, Billerica, MA, USA	SCMC001	Epigro base media supplemented with 6 mM L-Glutamine, 0.002% EpiFactor O (cell media supplement)

			O in article) , 1.0 $\mu$ M Epinephrine, 0.4% EpiFactor P (cell media supplement P in article), 5 $\mu$ g/mL rh Insulin, 5 $\mu$ g/mL Apo- Transferrin, and 100 ng/mL Hydrocortisone Hemisuccinate in Collagen-1 coated culture flasks (BioCoat, Corning, Tewksbury, MA, USA).
Live Dead calcien and ethidium homodimer	Invitrogen, Burlington, ON, Canada		
MesoScale Discovery (MSD) QuickPlex SQ 120 instrument	Rockville, MD, USA		
MSD Human Proinflammatory Panel II (4-Plex) V-Plex assay	Rockville, MD, USA		
Penicillin Streptomycin	Gibco	15140-122	100x concentration so add 1 mL to each 99 mL of media
Primary Corneal Epithelial Cells	Millipore, Billerica, MA, USA	SCCE016	
SpectraMax fluorescence multi-well plate reader	Molecular Devices, Sunnyvale, CA, USA		
TrypLE Express (cell disassociation solution)	Fisher Scientific	12605036	