

Materials List for:

Eye Irritation Test (EIT) for Hazard Identification of Eye Irritating Chemicals using Reconstructed Human Cornea-like Epithelial (RhCE) Tissue Model

Yulia Kaluzhny¹, Helena Kandárová², Laurence d'Argembeau-Thornton¹, Paul Kearney¹, Mitchell Klausner¹

¹MatTek Corporation

²MatTek In Vitro Life Science Laboratories

Correspondence to: Yulia Kaluzhny at ykaluzhny@mattek.com

URL: <http://www.jove.com/video/52979>

DOI: [doi:10.3791/52979](https://doi.org/10.3791/52979)

Materials

Name	Company	Catalog Number	Comments
Humidified incubator (37±1°C, 5±1% CO ₂ , 90±10% humidity)			Incubating tissues prior to and during assays
Laminar flow hood			Safe work under sterile conditions
Vacuum pump (optional)			Aspirating medium and solutions
Plate-reader photometer (for 96-well plates)			Reading OD
Plate shaker			Extraction of formazan
Sterile, blunt-edged forceps			Handling tissue inserts
Stop-watches			Timing of application of test materials and other timed steps in the protocol
Water bath (37°±1)			Warming media and MTT solution
Mortar and pestle			Grinding granular solids
Positive displacement pipette (50 µl)			Application of viscous and semi-solid materials and suspensions
Adjustable pipettes (200 µl – 2 ml)			Application of liquid materials, assay medium and MTT
Pre-sterilized tips (200 µl and 20 µl)	Rainin	HR-200F and HR-20F (or equivalent)	Application of liquid materials, assay medium and MTT
Wide orifice pre-sterilized tips (250 µl)	Rainin	Cat#HR-250WS (or equivalent)	Application of viscous and semi-solid materials and suspensions
8 oz/220 ml specimen containers	Falcon	3540200 (or equivalent)	Rinsing tissues
Sterile single-use syringes (e.g. 1 ml tuberkulin syringe Omnifix-F)	B. Braun Melsungen AG	9161406V	Delivery of ~50 mg solid materials (optional)
Ted Pella micro spatula/spoon	Ted Pella Inc.	13504 (or equivalent, sharp spoon or bone curette, e.g. Aesculap, No: FK 623)	Delivery of ~50 mg solid materials
Sterile deionized water, tissue culture grade (quality biological or equivalent)			Use as NC
96-well flat bottom plates	Falcon (or equivalent)		For reading OD
Cotton tip swaps (sterile)			For drying the tissue surface (optional)
Adhesive tape or Parafilm			Covering plates during formazan extraction
MTT-100 assay kit			Contains MTT-Thiazolyl Blue Tetrazolium Bromide reagent

			(Sigma #M-5655) and isopropanol extract.
Sealed 24-well plate of EpiOcular tissues (OCL-200)	MatTek		Contains 24 tissues of cell culture inserts, package on agarose
EpiOcular Assay Medium	MatTek	OCL-200-ASY	DMEM based medium
6-Well Plates	Falcon		Used for maintaining tissues during assay protocol
12-Well Plates	Falcon		Used during assay protocol
24-Well Plates	Falcon		Used to perform MTT assay
Methyl Acetate	Sigma-Aldrich	Cat# 186325 (CAS#79-20-9)	Used as PC in the assay