

Materials List for

# CAM-Delam Assay to Score Metastatic Properties by Quantifying Delamination and Invasion Capacity of Cancer Cells

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jove.com/video/64025

## Materials

Name	Company	Catalog Number	Comments
<b>EQUIPMENT</b>			
Centrifuge	Rotanta 480 R, Hettich zentrifugen		
Countess II FL Automated Cell Counter	Invitrogen		
Cryostat	HM 505 E, Microm		
Digital camera	Nikon DS-Ri1		
Dissection Microscope	Leica M10		For CAM-sample dissection and positioning in molds
Egg incubator	Fiem		Many other sources are available. Must be cleaned and sterilized with 70 % ethanol before each use
Epifluorescence microscope	Nikon Eclipse, E800		Equipped with a digital camera, for scoring delamination and cell invasion.
Fine forceps	Many sources are available		Must be sterilized before use
Freezer -80 °C	Thermo Fisher Scientific	8600 Series	Model 817CV
Inverted microscope	Nikon Eclipse TS100		For cell culture work
Scissors (small)	Many sources are available		Must be sterilized before use
<b>MATERIALS</b>			
anti-Laminin-111	Sigma-Aldrich	L9393	Primary anti-rabbit antibody (1:400)
anti-rabbit Cy3	Jackson Immuno Research	111-165-003	Secondary antibody (1:400)
anti-von Willebrand Factor	DAKO	P0226	Primary antibody (1:100)
Cobalt(II) chloride	Sigma-Aldrich	232696-5G	<b>CAUTION:</b> moderate toxicity chemical. Handle with care only in fume hood. Follow manufacturers instructions
DAPI	Sigma-Aldrich	D9542-10MG	4',6-diamidino-2-phenylindole, dihydrochloride (1:400)

Fertilized chicken eggs	Strömbäcks Ägg, Vännäs, Sweden		Any local egg supplier
Fetal bovine serum	Life Technologies	10500-064	
Fluorescence mounting medium	Allent Technologie	S302380-2	Avoid bubble formation when mounting. Allow to dry at +4 °C
Glass chambers for silicon rings	Many sources are available		We used 15 mL glass chambers. Around 20 silicon rings fit in one chamber. Avoid crowding, since the rings may stick together and aggravate work.
Glass coverslips	VWR International	631-0165	
GM6001 MMP Inhibitor	Sigma-Aldrich	CC1010	
Microscope slides for immunohistochemistry	Fisher Scientific	10149870	
NEG-50 frozen medium	Cellab	6506	
Paraformaldehyde	Sigma-Aldrich	30525-89-4	<b>CAUTION:</b> highly toxic. Handle with care only in fume hood, follow manufacturers instructions. Use all protective clothing.
Peel-A-Way Embedding Molds	Polysciences	18985	
Penicillin–streptomycin	Gibco	15070063	
Petri dishes	Sarstedt	83.3903	15 cm in diameter for cell culture
Plastic box	Esclain		
PureCol EZ Gel Collagen	Cellsystems	5074-35ML	5 mg/mL. Gelatinous material. Pipette very slowly and carefully to avoid cells being lost in the bubble formations.
RPMI medium	Thermo Fisher Scientific	21875034	
Silicon rings	VWR International	228-1580	Inner/outer diameter: 4/5 mm. Should be sterilized before use. Avoid repeated autoclaving of unused rings.
Trypan blue	Fisher Scientific	T10282	
Trypsin	Life Technologi	15400054	0.50%
Weighing boats	VWR International	611-0094	
<b>SOLUTIONS</b>			
Collagen-RPMI media mixture (1 mL)			Complete RPMI Media 750 µL
			PureCol EZ Gel Collagen 250 µL
			Mix and use immediately
Complete RPMI media (500 mL)			RPMI Media 445 mL
			FBS 50 mL
			Penicillin–streptomycin 5mL
			Store at 4 °C
PB (0.2 M; 1 000 mL)			Na <sub>2</sub> HPO <sub>4</sub> (MW 141.76) 21.9 g
			NaH <sub>2</sub> PO <sub>4</sub> (MW 137.99) 6.4 g
			add deionized water up to final volume of 1000ml
			Store in RT
PFA (4 %) in 0.1 M PB (100 mL)			Deionized water

			50 mL
			0.2 M PB 50 mL
			Paraformaldehyde (PFA) 4g
			heat to 60 °C in water bath
			add 5 M NaOH 25 µL
			Stir to dissolve the PFA powder
			Store at 4 °C
TBST (1 000 mL)			50mM Tris pH 7,4 50 mL
			150mM NaCl 30 mL
			0,1% Triton X-100 10 mL
			H <sub>2</sub> O (MQ) 900 mL
Trypsin (0.05 %; 10 mL)			1x PBS 9 mL
			10x Trypsin (0.5 %) 1 mL
			10 mL in total, Store at 4 °C