

Materials List for

Supervised Machine Learning for Semi-Quantification of Extracellular DNA in Glomerulonephritis

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jove.com/video/61180

Materials

Name	Company	Catalog Number	Comments
Bovine Serum Albumin	SIGMA	A2153	5% and 1% solutions are made up in PBS, can be made in bulk and frozen- discard once thawed.
Chicken anti Goat IgG (H+L) cross absorbed antibody Alexa Fluor 594	ThermoFisher Scientific	A-21468	Spin in mini centrifuge for 1 minute prior to use to avoid any free conjugate in your antibody cocktail
Chicken anti mouse IgG (H+L) cross absorbed antibody, Alex Fluor 647	ThermoFisher Scientific	A-121468	Spin in mini centrifuge for 1 minute prior to use to avoid any free conjugate in your antibody cocktail
Chicken anti rabbit IgG (H+L) Cross absorbed antibody Alexa Fluor 488	ThermoFisher Scientific	A-21441	Spin in mini centrifuge for 1 minute prior to use to avoid any free conjugate in your antibody cocktail
Chicken sera	SIGMA	C5405	Made up in 1%BSA/PBS
Coverslips 24 x60 mm	Azerscientific	ES0107222	#1.5 This is not standard thickness- designed for use in confocal microscopy
EDTA 10mM	SIGMA	E6758	Add TRIS and EDTA together in distilled water and pH to 9, for antigen retrieval, can be made up in a 10x Solution
Ethanol 30%, 70% and 100%	Chem Supply	UN1170	Supplied as 100% undenatured ethanol- dilute to 30% and 70% using distilled water
Formaldehyde, 4% (10% Neutral buffered Formalin)	TRAJAN	NBF-500	Kidney is put into a 5ml tube containing 3ml of formalin for 16 hours at RT, formalin should be used in a fume hood
Glass histology slides- Ultra Super Frost, Menzel Glaze, 25x75 x1.0mm	TRAJAN	J3800AM4	Using positive charged coated slides is essential. We do not recommend using poly-L-lysine for coating slides as tissue dislodges from slides during the antigen retrieval step
Goat anti human/mouse MPO antibody	R&D	AF3667	Aliquot and freeze at minus 80 degrees upon arrival
Histosol	Clini Pure	CPL HISTOSOL 08	Used neat, in 200ml staining rack containers, use in a fume hood

Hydrophobic pen	VECTOR Labs	H-400	Use to draw circle around kidney tissue
Mouse anti human/mouse Peptidyl arginase 4 (PAD4)	ABCAM	ab128086	Aliquot and freeze at minus 80 degrees upon arrival
Nikon C1 confocal scanning laser head attached to Nikon Ti-E inverted Microscope	Coherent Scientific		Aliquot and freeze at minus 80 degrees upon arrival
Phosphate Buffered Saline	SIGMA	P38135	0.01M PB/0.09% NaCl Make up 5L at a time
Pressure Cooker 6L Tefal secure 5 Neo stainless	Tefal	GSA-P2530738	Purchased at local homeware store
Prolong Gold DAPI	Life Technologies	P36962	Apply drops directly to coverslip
Rabbit anti human/mouse Beta Actin antibody	ABCAM	ab8227	Aliquot and freeze at minus 80 degrees upon arrival
Rabbit anti human/mouse H3Cit antibody	ABCAM	ab5103	Aliquot and freeze at minus 80 degrees upon arrival
Staining rack 24 slides	ProScitech	H4465	Staining rack chosen has to be able to withstand boiling under pressure and incubation in 60 degree oven
Sudan Black B	SIGMA	199664	0.3% Add 3g to a 1L bottle in 70% Ethanol, filter and protect from the light- stable for 6 months at room temperature
Tris 10mM	SIGMA	T4661	Add TRIS and EDTA together in distilled water and pH to 9, for antigen retrieval, can be made up in a 10x solution
Xylene	Trajan	XL005/20	Must be use used in a fume hood