

Materials List for:

Immunofluorescence Analysis of Endogenous and Exogenous Centromere-kinetochore Proteins

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Materials

Name	Company	Catalog Number	Comments
Lipofectamin 2000	Life Technologies/Invitrogen	11668	transfection reagent I
Lipofectamin RNAiMAX	Life Technologies/Invitrogen	13778	transfection reagent II
Opti-MEM I	Life Technologies/Invitrogen	31985	Reduced serum media, warm in 37 °C water bath before use
High-glucose DMEM (Dulbecco's modified Eagle's medium)	Life Technologies/BioWhittaker	12-604	high-glucose DMEM, warm in 37 °C water bath before use
Fetal Bovine Serum, certified, heat inactivated, US origin	Life Technologies/Gibco	10082	FBS (fetal bovine serum)
Poly-L-Lysine SOLUTION	SIGMA-SLDRICH	P 8920	Poly-L-Lysine, 0.1% w/v, in water
UltraPure Distilled Water	Life Technologies/Invitrogen/Gibco	10977	Sterile tissue culture grade water
Micro Cover glass (22 mm x 22 mm)	Surgipath	105	Cover glass (22 mm x 22 mm)
6 Well Cell Culture Cluster	Fisher/Corning Incorporated	07-200-83	6-well polystyrene plate
Penicillin, Streptomycin; Liquid	Fisher/Gibco	15-140	Penicillin-streptomycin
PAP PEN	Binding Site	AD100.1	Hydrophobic barrier pen (for a water repellent barrier in immunofluorescent staining)
Paclitaxel (Taxol)	SIGMA-SLDRICH	T7402	Taxol for mitotic cell analysis
TN-16, microtubule inhibitor (TN16)	Enzo Life Sciences	BML-T120	TN16 for mitotic cell analysis
BSA (bovine serum albumin)	SIGMA-SLDRICH	A7906	Blocking reagent
Triton X-100	SIGMA-SLDRICH	T8787	Detergent for permeabilization
Paraformaldehyde	SIGMA-SLDRICH	P6148	Fixation reagent
DAPI	SIGMA-SLDRICH	D9542	For nuclear staining
p-phenylenediamine	SIGMA-SLDRICH	P6001	For mounting medium
VWR Micro Slides, Frosted	VWR International	48312-013	Micro slides
Anti-CENP-A antibody	Stressgen/Enzo Life Sciences	KAM-CC006	Mouse monoclonal antibody; dilution ratio of 1:100 (IIF), 1:5000 (WB)
Anti-CENP-B antibody	Novus Biologicals	H00001059-B01P	Mouse monoclonal antibody; dilution ratio of 1:200 (IIF, methanol/acetone fixation)-1:400 (IIF, paraformaldehyde fixation)
Anti-CENP-B antibody	abcam	ab25734	Rabbit polyclonal antibody; dilution ratio of 1:200 (IIF, methanol/acetone fixation)-1:400 (IIF, paraformaldehyde fixation)
Anti-centromere antibody (ACA)	Fitzgerald Industries International, Inc.	90C-CS1058	Human centromere antiserum; dilution ratio of 1:2000 (IIF)

Anti-CENP-H antibody	Bethyl Laboratories	BL1112 (A400-007A)	Rabbit polyclonal antibody; dilution ratio of 1:200 (IIF)
Anti-CENP-H antibody	BD	612142	Mouse monoclonal antibody; dilution ratio of 1:200 (IIF)
Anti-CENP-I antibody	N/A, Dr. Katusmi Kitagawa	N/A, Dr. Katusmi Kitagawa	Rabbit polyclonal antibody; dilution ratio of 1:1000 (IIF); Niikura et al., Oncogene, 4133-4146 (2006)
Anti-KNL1 antibody	Novus Biologicals	NBP1-89223	Rabbit polyclonal antibody; dilution ratio of 1:100 (IIF)
Anti-Hec1 antibody	Novus Biologicals / GeneTex	NB 100-338 / GTX70268	Mouse monoclonal antibody; dilution ratio of 1:100 (IIF)
Anti-Hec1 antibody	GeneTex	GTX110735	Rabbit polyclonal antibody; dilution ratio of 1:100 (IIF)
Anti-Ska1 antibody	abcam	ab46826	Rabbit polyclonal antibody; dilution ratio of 1:100 (IIF)
Anti-Flag antibody	SIGMA-ALDRICH	F3165	Mouse monoclonal antibody; dilution ratio of 1:1000 (IIF), 1:5000 (WB)
Anti-Flag antibody	SIGMA-ALDRICH	F7425	Rabbit polyclonal antibody; dilution ratio of 1:1000 (IIF), 1:5000 (WB)
Anti-CUL4A antibody	N/A, Dr. Pradip Raychaudhuri	N/A, Dr. Pradip Raychaudhuri	Rabbit polyclonal antibody; dilution ratio of 1:3000 (WB); Shiyonov et al., The Journal of biological chemistry, 35309-35312 (1999)
Anti-RBX1 antibody	Cell Signaling	4397	Rabbit polyclonal antibody; dilution ratio of 1:2000 (WB)
Anti-GAPDH antibody	Chemicon	MAB374	Mouse monoclonal antibody; dilution ratio of 1:5000 (WB)
Alexa Fluor 488 Goat Anti-Mouse IgG	Life Technologies/Invitrogen	A11001	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
Alexa Fluor 594 Goat Anti-Mouse IgG	Life Technologies/Invitrogen	A11005	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
Alexa Fluor 488 Goat Anti-Rabbit IgG	Life Technologies/Invitrogen	A11008	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
Alexa Fluor 594 Goat Anti-Rabbit IgG	Life Technologies/Invitrogen	A11012	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
Non fat powdered milk (approved substitution for carnation powdered milk)	Fisher Scientific	NC9255871 (Reorder No. 190915; Lot# 90629)	Skim milk
Leica DM IRE2 motorized fluorescence microscope	Leica		motorized fluorescence microscope
HCX PL APO 63x oil immersion lens	Leica	LEICA HCX PL APO NA 1.40 OIL PH 3 CS	63X oil immersion lens
HCX PL APO 100x oil immersion lens	Leica	LEICA HCX PL APO NA 1.40 OIL PHE	100X oil immersion lens
Leica EL6000 compact light source	Leica		External compact light source for fluorescent excitation
ORCA-R2 Digital CCD camera	Hamamatsu	C10600-10B	digital CCD camera
Openlab version 5.5.2 Scientific Imaging Software	Perkin Elmer/Improvision		For image observation, acquisition, quantification, and analysis
Velocity version 6.1.1 3D Image Analysis Software	Perkin Elmer/Improvision		For image observation, acquisition, quantification, and analysis

Complete EDTA-free protease inhibitor cocktail	Roche	11873580001/11836170001	Protease inhibitor cocktail tablets
PlusOne 2-D Quant Kit	Amersham Biosciences	80-6483-56	Commercial protein assay reagent I for measurement of protein concentration (compatible with 2% SDS)
Bio-Rad Protein Assay	Bio-Rad	500-0006	Commercial protein assay reagent II for measurement of protein concentration (compatible with 0.1% SDS)
Immobilon-FL	EMD Millipore	IPFL00010	PVDF membrane for transferring
IRDye 800CW Goat Anti-Mouse IgG	LI-COR Biosciences	926-32210	IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:20000 (IIF)
IRDye 680 Goat Anti-Rabbit IgG	LI-COR Biosciences	926-32221	IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:20000 (IIF)
Goat anti-Mouse IgG DyLight 549	Fisher Scientific	PI35507	DyLight-conjugated secondary antibody/IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:20000 (IIF)
Goat anti-Rabbit DyLight 649	Fisher Scientific	PI35565	DyLight-conjugated secondary antibody/IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:20000 (IIF)
Goat anti-mouse IgG-HRP	Santa Cruz	SC-2005	HRP-conjugated secondary antibody/DyLight-conjugated secondary antibody/IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:10000 (IIF)
Goat anti-rabbit IgG-HRP	Santa Cruz	SC-2004	HRP-conjugated secondary antibody/DyLight-conjugated secondary antibody/IR fluorescent dye-conjugated secondary antibody (Affinity-purified secondary antibody); dilution ratio of 1:10000 (IIF)
Openlab version 5.5.2. Scientific Imaging Software	Improvision/PerkinElmer		Software A
Volocity version 6.3 3D Image Analysis Software (Volocity Acquisition)	PerkinElmer		Software B1
Volocity version 6.3 3D Image Analysis Software (Volocity Quantification)	PerkinElmer		Software B2
Branson SONIFIER 450			Sonicator
Branson Ultrasonics sonicator Microtip Step, Solid, Threaded 9.5 mm	VWR Scientific Products Inc.	33995-325	Disruptor horn for sonication
Branson Ultrasonics sonicator Microtip Tapered 6.5 mm	VWR Scientific Products Inc.	33996-185	Microtip for sonication
Odyssey CLx Infrared imaging System	LI-COR Biosciences		Infrared imaging system for immunoblot detection

Image Studio Analysis Software Ver 4.0	LI-COR Biosciences		Software C
Molecular Imager Versadoc MP4000 System	Bio-Rad		Chemiluminescence imager for immunoblot detection
Quantity One 1-D analysis software	Bio-Rad		Software D
SuperSignal West Femto Maximum Sensitivity Substrate	Thermo	34095	Ultra-sensitive enhanced chemiluminescent (ECL) substrate