

Materials List for:

Immunofluorescence Analysis of Stress Granule Formation After Bacterial Challenge of Mammalian Cells

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Materials

Name	Company	Catalog Number	Comments
Primary Antibodies			
eIF3b (N20), origin goat	Santa Cruz	sc-16377	Robust and widely used SG marker. Cytosolic staining allows cell delineation. Dilution 1:300
eIF3b (A20), origin goat	Santa Cruz	sc-16378	Same target as eIF3b (N20) and in our hands was identical to eIF3b (N20). Dilution 1:300
eIF3A (D51F4), origin rabbit (MC: monoclonal)	Cell Signaling	3411	Part of multiprotein eIF3 complex with eIF3b . Dilution 1:800
eIF4A1, origin goat	Santa Cruz	sc-14211	Recommended by (Ref # 13). Dilution 1:200
eIF4B, origin rabbit	Abcam	ab186856	Good stress granule marker in our hands. Dilution 1:300
eIF4B, origin rabbit	Cell Signaling	3592	Recommended by Ref # 13. Dilution 1:100
eIF4G, origin rabbit	Santa Cruz	sc-11373	Widely used SG marker. (Ref # 13): may not work well in mouse cell lines. Dilution 1:300
G3BP1, origin rabbit (MC: monoclonal)	BD Biosciences	611126	Widely used SG marker. Dilution 1:300
Tia-1, origin goat	Santa Cruz	sc-1751	Widely used SG marker. Can also be found in P bodies when SG are present (Ref # 13). Dilution 1:300
Alexa-conjugated Secondary Antibodies			
A488 anti-goat , origin donkey	Thermo Fisher	A-11055	Cross absorbed. Dilution 1:500
A568 anti-goat, origin donkey	Thermo Fisher	A-11057	Cross absorbed. Dilution 1:500
A488 anti-mouse, origin donkey	Thermo Fisher	A-21202	Dilution 1:500
A568 anti-mouse, origin donkey	Thermo Fisher	A10037	Dilution 1:500
A647 anti-mouse, origin donkey	Thermo Fisher	A31571	Dilution 1:500
A488 anti-rabbit, origin donkey	Thermo Fisher	A-21206	Dilution 1:500
A568 anti-rabbit, origin donkey	Thermo Fisher	A10042	Dilution 1:500
Other Reagents			
<i>Shigella flexneri</i>			Available from various laboratories by request
Tryptone Casein Soya (TCS) broth	BD Biosciences	211825	Standard growth medium for <i>Shigella</i> , application - bacterial growth

TCS agar	BD Biosciences	236950	Standard growth agar for Shigella, application - bacterial growth
Congo red	SERVA Electrophoresis GmbH	27215.01	Distrimination tool for Shigell that have lost the virulence plasmid, application - bacterial growth
Poly-L-Lysine (PLL)	Sigma-Aldrich	P1274	Useful to coating bacteria to increase infection, application - infection
Gentamicin	Sigma-Aldrich	G1397	Selective killing of extracellular but not cytosolic bacteria, application - infection
HEPES	Life Technologies	15630-056	PH buffer useful when cells are incubated at RT, application - cell culture
Dulbecco's Modified Eagle Medium (DMEM)	Life Technologies	31885	Standard culture medium for HeLa cells, application - cell culture
Fetal Calf Serum (FCS)	Biowest	S1810-100	5% supplementation used for HeLa cell culture medium, application - cell culture
Non-Essential Amino Acids (NEAA)	Life Technologies	11140	1:100 dilution used for HeLa cell culture medium, application - cell culture
DMSO	Sigma-Aldrich	D2650	Reagent diluent, application - cell culture
Sodium arsenite	Sigma-Aldrich	S7400	Potent stress granule inducer (Note: highly toxic, special handling and disposal required), application - stress inducer
Clotrimazole	Sigma-Aldrich	C6019	Potent stress granule inducer (Note:health hazard, special handling and disposal required), application - stress inducer
Paraformaldehyde (PFA)	Electron Microscopy Scences	15714	4% PFA is used for standard fixation of cells, application - fixation
Triton X-100	Sigma-Aldrich	T8787	Used at 0.03% for permeabilizationof host cells before immunofluorescent staining, application - permeabilization
A647-phalloidin	Thermo Fisher	A22287	Dilution is at 1:40, best added during secondary antibody staining, application - staining
DAPI	Sigma-Aldrich	D9542	Nucleid acid stain used to visualize both the host nucleus and bacteria, application - staining
Parafilm	Sigma-Aldrich	BR701501	Paraffin film useful for immunofluorescent staining of coverslips, application - staining
Prolong Gold	Thermo Fisher	36930	Robust mounting medium that works well for most fluorophores , application - mounting
Mowiol	Sigma-Aldrich	81381	Cheap and robust mounting medium that works well for most fluorophores, application - mounting
24-well cell culture plate	Sigma-Aldrich	CLS3527	Standard tissue culture plates, application - cell culture

12 mm glass coverslips	NeuVITRO	1001/12	Cell culture support for immunofluorescent applications, application - cell support
forceps	Sigma-Aldrich	81381	Cheap and robust mounting medium that works well for most fluorophores, application - mounting
Programs and Equipment			
Prism	GraphPad Software		Data analysis and graphing program with robust statistical test options, application - data analysis
Leica SP5	Leica Microsystems		Confocal microscope, application - image acquisition
Imaris	Bitplane		Professional image analysis program, application - data analysis
Excel	Microsoft		Data analysis and graphing program, application - data analysis