

Tyramide Signal Amplification for the Immunofluorescent Staining of ZBP1-dependent Phosphorylation of RIPK3 and MLKL After HSV-1 Infection in Human Cells

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Materials

Name	Company	Catalog Number	Comments
Antibodies			
Anti-rabbit HRP	Agilent Technologies Belgium	K4002	Envision+ System-HRP Labelled Polymer anti-rabbit
Goat anti-mouse DyLight 633	ThermoFisher	35513	Secondary antibody
HSV-1 ICP0	Santa Cruz	sc-53070	Mouse anti-ICP0(HSV-1) antibody
IAV-PR8 mouse serum	In house production	xx	Mouse anti-IAV-PR8 polyclonal antibody
pMLKL	Abcam	ab187091	Rabbit anti-MLKL-phospho S358 antibody
pRIPK3	Abcam	ab209384	Rabbit anti-RIPK3-phospho S227 antibody
Fluorophores			
DAPI	ThermoFisher	D21490	To visualise the nucleus of the cells
Streptavidin coupled to Alexa Fluor 568	ThermoFisher	S11226	To visualise biotin molecules
Compounds			
30% H ₂ O ₂	Sigma	H1009	Oxidising substrate, necessary for HRP activity
4% PFA	SANBIO	AR1068	To fix/crosslink the cells
Biotinyl-tyramide	R&D Systems	6241	To amplify signal, HRP substrate
BV-6	Selleckchem	S7597	BV6 IAP Inhibitor
			For cell culture: to detach the cells
			8.0 g/L NaCl
			0.4 g/L disodium salt of EDTA
EDTA 0.04%	In house formulation		1.1 g/L Na ₂ HPO ₄
			0.2 g/L NaH ₂ PO ₄
			0.2 g/L KCl

			0.2 g/L Glucose
Fetal Bovine serum	TICO	FBS EU XXX	For cell culture, maintaining cell culture; lot number: 90439
GSK'840	Aobious	AOB0917	RIPK3 kinase inhibitor
L-Glutamine	Sigma-Aldrich	G7513	For cell culture, maintaining cell culture
MAXblock	Active Motif	15252	Blocking solution
PBS	Gibco	10444402	
Sodium pyruvate	Sigma-Aldrich	S8636	For cell culture, maintaining cell culture
TNF- α	In house production	-	Signaling molecule, able to trigger cell death in combination with BV6 and zVAD
Triton X-100	Sigma Aldrich	T8787-50ML	To permeabilise the cells
Trypan blue	Merck	11732	For cell counting, used as live/dead marker at 0,1%
Trypsine	Sigma-Aldrich	T4424	For cell culture: to detach the cells
zVAD	Bachem	BACE4026865.0005	Z-Val-Ala-DL-Asp-fluoromethylketone
Material			
μ -Slide 8 well high glass bottom	iBidi	80807	To culture the cells
Cotton Preping Balls-size medium	Electron Microscopy Sciences	71001-10	To clean the objectives
Immersol 518 F / 30 °C	ZEISS	444970-9000-000	To visualise the sample at high magnifications
Lens Cleaner	ZEISS	000000-0105-200	To clean the objectives
LSM880 Fast Airyscan confocal microscope			To visualise the sample
Software			
Excel	Office	xx	To process the data
Prism 9	Graphpad	xx	To analyse the data- statistical testing and graph generation
Volocity 6.3	Volocity	xx	To perform quantifications
Zen black	ZEISS	xx	To aquire and process images
Zen blue	ZEISS	xx	To visualise images
Viruses			
HSV-1 (mutRHIM) F strain	produced by Dr. Jiahuai Han	in house replication	HSV-1 as a trigger for necroptosis; RHIM core domain of UL39/ICP6 is mutated (VQCG>AAAA)
HSV-1 (WT) F strain	Produced by Dr. Jiahuai Han	in house replication	HSV-1 (WT) as a negative control for necroptosis induction (ICP6 inhibition)
IAV PR8	in house stock	in house replication	IAV as a trigger for necroptosis