

Materials List for

# Evaluation of Substrate Ubiquitylation by E3 Ubiquitin-ligase in Mammalian Cell Lysates

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## Citation

dos Passos, P.M.S., Spagnol, V., de Correia, C.R., Teixeira, F.R. Evaluation of Substrate Ubiquitylation by E3 Ubiquitin-ligase in Mammalian Cell Lysates. *J. Vis. Exp.* (183), e63561, doi:10.3791/63561 (2022).

## Date Published

May 10, 2022

## DOI

10.3791/63561

## URL

jove.com/video/63561

## Materials

Name	Company	Catalog Number	Comments
1.5 mL microtube	Axygen	PMI110-06A	
100 mm TC-treated culture dish	Corning	430167	
15 mL tube	Corning	430766	
96-well plate	Cralplast	655111	
Agarose-anti-HA beads	Sigma-Aldrich	E6779	
Anti Mouse antibody	Seracare	5220-0341	Goat anti-Mouse IgG
Anti Rabbit antibody	Seracare	5220-0337	Goat anti-Rabbit IgG
Anti-Actin antibody	Sigma-Aldrich	A3853	Dilution used: 1:2000
Anti-Fbxo7 antibody	Sigma-Aldrich	SAB1407251	Dilution used: 1:1000
Anti-HA antibody	Sigma-Aldrich	H3663	Dilution used: 1:1000
Anti-Myc antibody	Cell Signalling	2272	Dilution used: 1:1000
Bradford reagent	Sigma-Aldrich	B6916-500ML	
BSA	Sigma-Aldrich	A9647-100G	Bovine Serum Albumin
Cell incubator	Nuaire	NU-4850	
Centrifuge	Eppendorf	5804R	500 x g for 5 min
ChemiDoc	BioRad		
Digital pH meter	Kasvi	K39-2014B	
Dulbecco's Modified Eagle's Medium	Corning	10-017-CRV	High glucose
Fetal bovine serum	Gibco	F4135	Filtrate prior use
HA peptide	Sigma-Aldrich	I2149	
HEK293T cells	ATCC	CRL-3216	
Hepes	Gibco	15630080	
KCl	VWR Life Science	0365-500G	
Kline rotator	Global Trade Technology	GT-2OIBD	
MG-132	Boston Biochem	I-130	
Microcentrifuge	Eppendorf	5418R	

Na <sub>3</sub> VO <sub>4</sub> (Ortovanadato)			
NaF			
Nitrocellulose blotting membrane	GE Healthcare	10600016	
NP40 (IGEPAL CA-630)	Sigma-Aldrich	I8896-100ML	
Optical microscope	OPTIKA microscopes	SN510768	
Opti-MEM	Gibco	31985-070	
pcDNA3	Invitrogen	V79020	For mammalian expression
pcDNA3-2xFlag-Fbxo7	Kindly donated by Dr. Marcelo Damário		Tag 2xFlag (N-terminal). Restriction enzymes: EcoRI and XhoI
pcDNA3-2xFlag-Fbxo7-ΔF-box	Kindly donated by Dr. Marcelo Damário		Tag 2xFlag (N-terminal). Restriction enzymes: EcoRI and XhoI. Δ335-367
pcDNA3-UXTV2-HA	Kindly donated by Dr. Marcelo Damário		Tag HA (C-terminal). Restriction enzymes: EcoRI and XhoI
pCMV-6xHis-Myc-Ubiquitin	Kindly donated by Dr. Marcelo Damário		Tag 6x-His-Myc (N-terminal). Restriction enzymes: EcoRI and KpnI
Pen Strep Glutamine 100x	Gibco	10378-016	
Phosphate buffered saline 10x	AccuGENE	51226	To obtain a 1x PBS, dilute the 10x PBS into ultrapure water
Polyethylenimine (PEI)	Sigma-Aldrich	9002-98-6	
Ponceau S	VWR Life Science	0860-50G	
Protease inhibitor cocktail SIGMAFAST	Sigma-Aldrich	S8820	
Rocking Shaker	Kasvi	19010005	
SDS-PAGE system	BioRad	165-8004	
Solution Homogenizer	Phoenix Luferto	AP-22	
Trizma base	Sigma-Aldrich	T6066-500G	
Trypsine (TrypLe Express)	Gibco	12605-028	
Western Blotting Luminol Reagent	Santa Cruz Biotechnology	SC-2048	