

In Vitro Analysis of E3 Ubiquitin Ligase Function

 Leonie Müller¹, Carl Elias Kutzner^{1,2}, Vishnu Balaji¹, Thorsten Hoppe^{1,2}
¹Institute for Genetics and Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), University of Cologne

²Center for Molecular Medicine Cologne, University of Cologne

Corresponding Author

Thorsten Hoppe

thorsten.hoppe@uni-koeln.de

Citation

 Müller, L., Kutzner, C.E., Balaji, V., Hoppe, T. *In Vitro Analysis of E3 Ubiquitin Ligase Function. J. Vis. Exp.* (171), e62393, doi:10.3791/62393 (2021).

Date Published

May 14, 2021

DOI

10.3791/62393

URL

jove.com/video/62393

Materials

Name	Company	Catalog Number	Comments
Amershan Protran 0.1 µm NC	GE Healthcare	10600000	nitrocellulose membrane
Anti-CHIP	Cell Signaling	2080	Monoclonal rabbit anti-CHIP antibody, clone C3B6
Anti-MYC	Roche	OP10	Monoclonal mouse anti-MYC antibody, clone 9E10
Anti-ubiquitin	Upstate	05-944	Monoclonal mouse anti-Ub antibody, clone P4D1-A11
Apyrase	Sigma	A6535-100UN	
ATP (10x)	Enzo	12091903	
BSA	Sigma	A6003-10G	
EDTA	Roth	8043.2	
KCl	Roth	6781.1	
K ₂ HPO ₄	Roth	P749.2	
KH ₂ PO ₄	Roth	3904.1	
LDS sample buffer (4x)	novex	B0007	
L-Lysine	Sigma	L5501-5G	
MES	Roth	4256.4	
MeOH	VWR Chemicals	2,08,47,307	100%
Milchpulver	Roth	T145.3	
NaCl	Roth	P029.3	
NuPAGE Antioxidant	invitrogen	NP0005	
NuPAGE Transfer buffer (20x)	novex	NP0006-1	
Page ruler plus	Thermo Fisher	26619	Protein ladder
RotiBlock	Roth	A151.1	Blocking reagent
SDS (20%)	Roth	1057.1	
S1000 Thermal Cycler	Bio Rad	1852196	
Trans-Blot Turbo	Bio Rad	1704150EDU	Transfer system
Tris base	Roth	4855.3	
Tween 20	Roth	9127.2	
UbqH Enzyme Set	BostonBiochem	K-980B	E2 enzymes

Ubiquitin	BostonBiochem	U-100H	
Ubiquitin-activating enzyme E1	Enzo	BML-UW941U-0050	
Ubiquitylation buffer (10x)	Enzo	BML-KW9885-001	
Whatman blotting paper	Bio Rad	1703969	Extra thick filter paper