

Bioluminescent Optogenetics 2.0: Harnessing Bioluminescence to Activate Photosensory Proteins *In Vitro* and *In Vivo*

 Emmanuel L. Crespo^{1,2}, Andreas Bjorefeldt¹, Mansi Prakash¹, Ute Hochgeschwender^{1,2,3}
¹College of Medicine, Central Michigan University ²Biochemistry, Cell and Molecular Biology Program, Central Michigan University ³Neuroscience Program, Central Michigan University

Corresponding Author

Ute Hochgeschwender

hochg1u@cmich.edu

Citation

 Crespo, E.L., Bjorefeldt, A., Prakash, M., Hochgeschwender, U. Bioluminescent Optogenetics 2.0: Harnessing Bioluminescence to Activate Photosensory Proteins *In Vitro* and *In Vivo*. *J. Vis. Exp.* (174), e62850, doi:10.3791/62850 (2021).

Date Published

August 4, 2021

DOI

10.3791/62850

URL

jove.com/video/62850

Materials

Name	Company	Catalog Number	Comments
ABI 25W Deep Red 660 nm LED Light Bulb	Amazon		to be used with any lamp stand
Black Microcentrifuge Tubes, 0.5 mL, Argos Technologies	Fisher Scientific	03-391-166	
Black Microcentrifuge Tubes, 1.5 mL, Argos Technologies	Fisher Scientific	03-391-161	
Black Nylon, Polyurethane-Coated Fabric (1.5 m x 2.7 m) x 0.12 mm (thick)	THOR LABS	BK-5	
C120-Fluc			K. Gardner
CaCl ₂	Sigma	C8106; CAS: 10035-04-8	
Ca-FLARE protease, transcription factor and reporter		Addgene # 92214, 92213, 92202	A. Ting
CIB-VP64 (B1016)		Addgene # 92037	C. Tucker
CRY-GalΔDD (B1013)		Addgene # 92035	C. Tucker
CTZ	Prolume Inc. (NanoLight)	303	formulation for in vitro applications with <i>Gaussia</i> luciferases
CTZ (Water soluble native coelenterazine)	Prolume Inc. (NanoLight)	3031	formulation for in vivo applications with <i>Gaussia</i> luciferases
D-(+)-Glucose	Sigma	G8270; CAS: 50-99-7	
D-Luciferin, Potassium Salt	Gold Biotechnology	LUCK	
DMEM	Thermo Fisher	11960044	
D-PBS, no calcium, no magnesium	Thermo Fisher	14190144	
hCTZ	Prolume Inc. (NanoLight)	301	formulation for in vitro applications with <i>Oplophorus</i> luciferases
HEK293	ATCC	CRL-1573	
HeLa	ATCC	CCL-2	
HEPES	Sigma	H3375; CAS: 7365-45-9	

iCreV			A. Cetin and H. Zeng
In Vivo Imaging System (IVIS)	Perkin-Elmer	Lumina LT	
KCl	Sigma	P5405; CAS: 7447-40-7	
LED Array Driver	Amuza	LAD-1	
LED Array for Multiwell Plates	Amuza	LEDA-x	
Lipofectamine 2000 Reagent	Invitrogen	11668-019	Transfection reagent
Luminometer	Molecular Devices	SpectraMax L	
MgCl ₂ Hexahydrate	Sigma	M2670; CAS: 7791-18-6	
NaCl	Sigma	S7653; CAS: 7647-14-5	
NanoFuel Solvent	Prolume Inc. (NanoLight)	399	for dissolving CTZ preparations for in vitro use
NaOH	Sigma	221465; CAS: 1310-73-2	
NES-CRY2PHR-TevC		Addgene # 89877	H. Kwon
Opti-MEM	Thermo Fisher	11058021	transfection medium
PDL coated coverslips (12 mm, 15 mm, 18 mm)	Neuvitro Corporation	GG-12-PDL, GG-15-PDL , GG-18-PDL	
pGL2-GAL4-UAS-Luc		Addgene #33020	M. Walsh
Prizmatix USB Pulser TTL Generator for Optogenetics	Goldstone Scientific		
TM-CIBN-BLITz1-TetR-VP16		Addgene # 89878	H. Kwon
TrypLE Express	Gibco	12604-013	
Vehicle (Water-soluble carrier without CTZ)	Prolume Inc. (NanoLight)	3031C	control for in vivo applications with CTZ
VP-EL222			K. Gardner