

Analyzing Oxidative Stress in Murine Intestinal Organoids using Reactive Oxygen Species-Sensitive Fluorogenic Probe

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Citation

Stedman, A., Levy, A., Sansonetti, P.J., Nigro, G. Analyzing Oxidative Stress in Murine Intestinal Organoids using Reactive Oxygen Species-Sensitive Fluorogenic Probe. *J. Vis. Exp.* (175), e62880, doi:10.3791/62880 (2021).

Date Published

September 17, 2021

DOI

10.3791/62880

URL

jove.com/video/62880

Materials

Name	Company	Catalog Number	Comments
Mice			
Lgr5-EGFP-IRES-creERT2 (Lgr5-GFP)	The Jackson Laboratory		
Growth culture medium			
Advanced DMEM F12 (DMEM/F12)	ThermoFisher	12634010	
B-27 Supplement, minus vitamin A	ThermoFisher	12587010	Stock Concentration: 50x
GlutaMAX (<i>glutamine</i>)	ThermoFisher	35050038	Stock Concentration: 100x
Hepes	ThermoFisher	15630056	Stock Concentration: 1 M
Murine EGF	R&D	2028-EG-200	Stock Concentration: 500 µg/mL in PBS
murine Noggin	R&D	1967-NG/CF	Stock Concentration: 100 µg/mL in PBS
Murine R-spondin1	R&D	3474-RS-050	Stock Concentration: 50 µg/mL in PBS
N-2 Supplement	ThermoFisher	17502048	Stock Concentration: 100x
Penicillin-Streptomycin (P/S)	ThermoFisher	15140122	Stock Concentration: 100x (10,000 units/mL of penicillin and 10,000 µg/mL of streptomycin)
Material			
70 µm cell strainer	Corning	352350	
96-well round bottom	Corning	3799	
ball tip scissor	Fine Science Tools GMBH	14086-09	
CellROX® Deep Red Reagent	ThermoFisher	C10422	
DAPI (4',6-diamidino-2-phénylindole, dichlorhydrate) (<i>fluorogenic probe</i>)	ThermoFisher	D1306	stock at 10 mg/mL

DPBS 1x no calcium no magnesium (DPBS)	ThermoFisher	14190144	
FLuoroBrite DMEM (<i>DMEM no phenol red</i>)	ThermoFisher	A1896701	
Hoechst 33342	ThermoFisher	H3570	stock at 10 mg/mL
Matrigel Growth Factor Reduced, Phenol Red Free (<i>Basement Membrane Matrix</i>)	Corning	356231	once received thaw o/n in the fridge, keep for 1h on ice and, make 500 mL aliquots and store at -20 °C
μ-Slide 8 Well chambers	Ibidi	80826	
N-acetylcysteine (NAC)	Sigma	A9165	
tert-Butyl hydroperoxide (tBCHP)solution (70%wt. In H ₂ O ₂)	Sigma	458139	
TrypLE Express Enzyme (1X), no phenol red (<i>trypsin</i>)	ThermoFisher	12604013	
UltraPure 0.5 M EDTA, pH8.0	ThermoFisher	15575020	
Y-27632	Sigma	Y0503	Rock-inhibitor to be used to minimize cell death upon tissue dissociation
Programs and Equipment			
Attune NxT (Flow Cytometer)	ThermoFischer		Flow cytometer analyzer
Fiji/ImageJ	https://imagej.net/software/fiji/downloads		images generation
FlowJo	BD Bioscience		FACS analysis
Observer.Z1	Zeiss		confocal system
Opterra (swept-field confocal)	Bruker		confocal system
high speed EMCCD Camera Evolve Delta 512	Photometrics		confocal system
Prism	GraphPad Software		statistical analysis