

Neutrophil Lifespan Extension with CLON-G and an *In Vitro* Spontaneous Death Assay

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Materials

Name	Company	Catalog Number	Comments
0.2 µm syringe filter	Pall Corporation	4612	Filtrate prepared CLON-G components.
1.5 mL micro centrifuge tube	LABSELECT	MCT-001-150	Lab consumable.
15 mL Centrifuge Tubes	LABSELECT	CT-002-15	Lab consumable.
24 well cell culture plate	Falcon	351147	Neutrophil culture plate.
50 mL Centrifuge Tubes	LABSELECT	CT-002-50	Lab consumable.
BD LSRII	BD		Instrument for flow cytometry analysis of neutrophil death.
Calcium chloride (CaCl ₂)	Sigma Aldrich	C4901	Assitant of Annexin-V binding to phosphatidylserine.
Confocal microscope	Perkinelmer	UltraVIEW VOX	Instrument for fluorescent analysis of neutrophil death.
Confocal plate	NEST	801001-20mm	Lab consumable for fluorescent image assay.
Counting beads	Thermo Fisher	C36950	Quantification in flow cytometry analysis of neutrophil death.
DFO	Sigma Aldrich	D9533	Component of CLON-G. LMP inhibitor.
Dimethyl sulfoxide (DMSO)	Sigma Aldrich	D2650	Solvent for Q-VD-oph and Nec-1s.
Fetal Bovine Serum	Gibco	10099141C	Component of neutrophil culture basic medium. Nutrition supply.
FITC-Annexin-V	BD	51-65874X	Annexin-V can bind to phosphatidylserine of aged cells.This is at FITC channel.
Hsp70	Abcam	ab113187	Component of CLON-G. LMP inhibitor.
NAC	Sigma Aldrich	A9165	Component of CLON-G. Antioxidant.

Nec-1s	EMD Millipore	852391-15-2	Component of CLON-G. Necroptosis inhibitor.
Penicillin-Streptomycin Solution (PS)	Gibco	15070063	Component of neutrophil culture basic medium. Antibiotics to protect cells from bacteria contamination.
Propidium Iodide (PI)	BioLegend	421301	For neutrophil death assay. A small fluorescent molecule that binds to DNA but cannot passively traverse into cells that possess an intact plasma membrane.
Q-VD-oph	Selleck chem	S7311	Component of CLON-G. Pan-caspase inhibitor.
Recombinant Human Granulocyte Colony-stimulating Factor for Injection (CHO cell)(G-CSF)	Chugai Pharma China	GRANOCYTE	Component of CLON-G. Promote neutrophil survival through Akt pathway.
Round-Bottom Polystyrene Tubes	Falcon	100-0102	Lab consumable for flow cytometry analysis.
RPMI1640	Gibco	C11875500BT	Component of neutrophil culture basic medium.
Saline	LEAGENE	R00641	Solution for flow cytometry analysis of neutrophil death.
Sodium hydroxide (NaOH)	FENG CHUAN	13-011-00029	pH adjustment for NAC.
Wright-Giemsa Stain Solution	Solarbio	G1020	Neutrophil cytospin staining.