

# Standardized *In vitro* Assays to Visualize and Quantify Interactions between Human Neutrophils and *Staphylococcus aureus* Biofilms

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jove.com/video/63773

## Materials

Name	Company	Catalog Number	Comments
0.9% sodium chloride irrigation, USP	Baxter	2F7124	Endotoxin-free; Used for isolation of neutrophils
150 mL rapid-flow filter unit	Thermo Scientific	565-0020	
200 proof ethanol	VWR	89125-188	
3 mL syringe	BD	309657	Used for blood draw
50 mL conical centrifuge tubes	Thermo Scientific	339652	
60 mL syringe	BD	309653	Used for blood draw
Agar	Fisher Bioreagents	BP1423-2	
Alcohol swab	BD		Used for blood draw
Band-aids			Used for blood draw
BD Bacto Tryptic Soy Broth	BD	DF0370-07-5	Combine with 1.5% agar to make Tryptic Soy Agar
Cell counter	Bal Saupply	202C	
CellTracker blue CMCH	Invitrogen	C2111	Blue CMAC Dye (BCD)
Clear bottom 96-well flat bottom polystyrene plates	Costar	3370	
Cotton gauze	Fisherbrand	13-761-52	Used for blood draw
Crystal violet	Acros Organic	40583-0250	
Culture tubes	Fisherbrand	14-961-27	Borosilicate Glass 13 x 100 mm
D-(+)-glucose	Sigma	G-8270	
Dextran from <i>Leuconostoc</i> spp.	Sigma	31392-250G	Used for isolation of neutrophils
Dulbecco's phosphate buffered saline (DPBS) 1x	Gibco	14190-144	
Ethidium homodimer-1	Invitrogen	L3224 B	
Ficoll-Paque plus	Cytiva	17144003	Used for isolation of neutrophils (density gradient medium)
Hanks' balanced salt solution (HBSS) 1x	Corning cellgro	21-022-CV	without calcium, magnesium, and phenol red

Hemocytometer	Bright Line		
Heparin	Novaplus	NDC 63323-540-57	1000 USP units/mL, Used for blood draw
IMARIS 9.8	Oxford Instruments		Microscopy image analysis software
Luminol	Sigma	A8511-5G	
Minimal essential media (MEM) Alpha 1x	Gibco	41061-029	
Needle (23 G1)	BD	305145	Used for blood draw
Nikon Eclipse Ti2	Nikon		
NIS-Elements	Nikon		Quantification of dead neutrophils
Normal human serum	Complement Technology	NHS	
Petri Dish (100 x 15 mm)	VWR	25384-342	
Phorbol 12-myristate 13-acetate			
Poly-L-lysine solution	Sigma	P4707-50ML	
Sodium chloride	Fisher Bioreagents	BP358-10	Used for neutrophil isolation
SoftMax Pro Software	Molecular Devices		Microplate reader software used for data acquisition
SpectraMax i3x	Molecular Devices		Microplate reader
Sterile water for irrigation, USP	Baxter	2F7114	Endotoxin-free; Used for neutrophil isolation
Surflo winged infusion set	Terumo	SC*19BLK	19 G x 3/4", used for blood draw
Trypan blue stain (0.4%)	Gibco	15250-061	
Turnicate			Used for blood draw
UltraPure distilled water	Invitrogen	10977015	
White opaque 96-well plates	Falcon	353296	Tissue culture treated and flat bottom plate
μ-Slide VI 0.4	Ibidi	80601	μ-channel slide