

Materials List for:

A Rapid Image-based Bacterial Virulence Assay Using Amoeba

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Materials

Name	Company	Catalog Number	Comments
Reagents			
Bacto agar, dehydrated	BD Difco	214010	
Antibiotic-Antimycotic (100X)	Life Technologies	15240062	Aliquot < 1 mL and store at -20 °C
Calcein-acetoxymethyl ester (calcein-AM)	Life Technologies	C34852	Calcein Acetoxymethyl (AM)
Calcium chloride, anhydrous	Sigma-Aldrich	C1016	
D-Glucose	Fisher Chemical	D16500	Dextrose
Dimethyl sulfoxide	Sigma-Aldrich	D5879	
Folic acid	Sigma-Aldrich	F8758	
LB-Miller	BD Difco	244620	
Magnesium chloride	Sigma-Aldrich	M8266	
Yeast extract	Oxoid	LP0021	
Special peptone	Oxoid	LP0072	
Potassium hydroxide	Fisher Chemical	P250	
Potassium phosphate monobasic	Sigma-Aldrich	P0662	
Sodium phosphate dibasic heptahydrate	Fisher Chemical	S373	
Vitamin B12	Sigma-Aldrich	V2876	
Strains			
Dictyostelium discoideum	Siryaporn lab	Strain AX3 ¹⁸	
Escherichia coli	Siryaporn lab	Strain B/r ¹⁷	
Pseudomonas aeruginosa	Siryaporn lab	PA14	PA14 strain ¹⁹
Pseudomonas aeruginosa ΔlasR	Siryaporn lab	AFS20.1	PA14-derived strain ²⁰
Supplies			
0.22 μm filter	Millipore	SCGPT01RE	For filter sterilization
Conical tube, 15 mL	Corning	352097	
Glass storage bottles	Pyrex	13951L	250 mL, 500 mL, 1000 mL
Petri dish, 6 cm diameter	Corning	351007	60 x 15 mm polystyrene plates
Petri dish, 10 cm diameter	Fisher	FB0875712	100 x 15 mm polystyrene plates
Plastic containers with lid	Ziploc	2.57E+09	Square 3-cup containers
Glass plates	Bio-Rad	1653308	For preparing agar pads. Other glass plates may be used with similar dimensions.
Wooden sticks	Fisher	23-400-102	
Equipment			

Eclipse Ti-E microscope	Nikon	MEA53100	Microscope setup
10X Plan Fluor Ph1 objective 0.3 NA	Nikon	MRH20101	Microscope setup
Fluorescence excitation source	Lumencor	Sola light engine	Microscope setup
Fluorescence filter set	Semrock	LED-DA/FI/TX-3X3M-A-000	Microscope setup
Orca Flash 4.0 V2 Camera	Hamamatsu	77054098	Microscope setup
ImageJ	NIH	v. 1.49	Software for image analysis
MATLAB	Mathworks	R2013	Software for image analysis
Orbital shaker incubator	VWR	89032-092	For growth of bacteria at 37 °C
Platform shaker	Fisher	13-687-700	For growth of amoebae at 22 °C
Spectrophotometer	Biochrom	Ultrospec 10	
Undercounter refrigerated incubator	Fisher	97990E	For growth of amoebae at 22 °C
Isotemp waterbath	Fisher	15-462-21Q	For cooling media to 55 °C