

Materials List for:

High Throughput, Real-time, Dual-readout Testing of Intracellular Antimicrobial Activity and Eukaryotic Cell Cytotoxicity

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Materials

Name	Company	Catalog Number	Comments
J774A.1 cells	American Type Culture Collection	TIB-67	Host cell
ACES	Sigma-Aldrich	A9758	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Yeast extract, ultrafiltered	Becton-Dickinson/Difco	210929	For making buffered charcoal yeast extract agar and buffered yeast extract medium; lower grades may cause impaired growth and/or alter sensitivity of Legionella to growth inhibitors
Alpha-ketoglutaric acid, monopotassium salt	Sigma-Aldrich	K2000	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Sodium pyruvate	Sigma-Aldrich	P5280	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Potassium phosphate, dibasic	Thermo Fisher Scientific	P288-500	For making buffered charcoal yeast extract agar and buffered yeast extract medium
L-cysteine	Sigma-Aldrich	C-7755	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Ammonium iron(III) citrate	Sigma-Aldrich	F5879	For making buffered charcoal yeast extract agar and buffered yeast extract medium; ferric pyrophosphate may be used instead but is more difficult to weigh accurately
Potassium hydroxide solution, concentrated	Thermo Fisher Scientific	SP236-500	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Deionized water	N/A	N/A	For making buffered charcoal yeast extract agar and buffered yeast extract medium
Thymidine (tissue culture grade)	Sigma-Aldrich	T1895	For supplementing both RPMI 1640 and buffered yeast extract agar/medium — lower grade thymidine may be used for the latter, but may cause impaired cell growth and/or cell death in RPMI 1640
RPMI 1640, standard formulation	Corning via Thermo Fisher Scientific	10-040-CV	For growing J774A.1 cells prior to plating; includes 2 mM L-glutamine
RPMI 1640 lacking phenol red	Corning via Thermo Fisher Scientific	17-105-CV	For plating J774A.1 cells in 384 well dishes (not suitable for growth)

			prior to plating); also lacks L-glutamine — supplement to 2 mM before use
L-glutamine, 200 mM in 0.85% NaCl (tissue culture grade)	HyClone via Thermo Fisher Scientific	SH30034.02	For supplementing RPMI 1640 lacking L-glutamine, to 2 mM final concentration
Iron-supplemented calf serum	Gemini Bioproducts	100-510	For supplementing RPMI 1640, to 9.1% final concentration
Trypan Blue solution	Sigma-Aldrich	T8154	For staining for J774A.1 cell death determination while counting cell density
SYTOX Green, 5 mM solution in DMSO	Thermo Fisher Scientific	S7020	For staining for J774A.1 cell death determination by fluorescence reading or epifluorescence microscopy (in conjunction with orange-red or far red fluorescent bacteria). Use at 125 nM final concentration.
Cell culture incubator	Thermo Fisher Scientific	13-255-26	For incubation of J774A.1 cells (both before and after infection); can also be used for incubation of bacteria, or a standard atmosphere incubator can be used instead)
Orbital shaker	BellCo Glass	7744-01010	For shaking incubation of J774A.1 cells before infection; fits inside cell culture incubator; includes shaker base 7744-01000 and tray 7740-01010 (these are also available separately)
Shaker flasks (250 ml)	ChemGlass Life Sciences	CLS-2038-04	For shaking incubation of J774A.1 cells before infection
Shaker clamps for flasks (250 ml)	BellCo Glass	7744-16250	For shaking incubation of J774A.1 cells before infection
Shaker flasks (1,000 ml)	ChemGlass Life Sciences	CLS-2038-07	For shaking incubation of J774A.1 cells before infection
Shaker clamps for flasks (1,000 ml)	BellCo Glass	7744-16100	For shaking incubation of J774A.1 cells before infection
Sponge foam caps for flasks (250 ml-1,000 ml)	ChemGlass Life Sciences	CLS-1490-038	For shaking incubation of J774A.1 cells before infection; reduces risk of contamination relative to standard metal caps
MultiDrop Combi programmable multichannel peristaltic pump	Thermo Fisher Scientific	5840300	For dispensing J774A.1 cells, medium, and bacterial suspension containing fluorophores to large numbers of 384 well dishes
Combi standard bore manifold	Thermo Fisher Scientific	24072670	Default predisperse volume of 20 μ l is insufficient to compensate for settling — increase to 80 μ l
White 384 well dishes treated for tissue culture	Corning	3570	For reading luminescence and fluorescence; Greiner catalog # 781080 also tested successfully
DMSO (tissue culture grade, in sealed ampoules)	Sigma-Aldrich	D2650	For dissolving positive control and test compounds
Azithromycin	Sigma-Aldrich	PHR1088	Antibiotic positive control
Saponin (from Quillaja bark)	Sigma-Aldrich	S-4521	Cytotoxicity positive control
Multichannel pipettor	Thermo Fisher Scientific	Finnpipette	For transfer of fixed amounts of positive control compounds; pipettor must have digital dispensing with detents to enable repetitive fixed volume dispensing

Epson pin transfer robot	Epson/ICCB-L	(Custom equipment)	For transfer of fixed amounts of test compounds from library arrays
D300 digital dispensing system	Hewlett-Packard via Tecan	D300	For transfer of variable amounts of test compounds ranging from 11 picoliters to 10 μ l
T8+ cartridges for D300 digital dispensing system	Hewlett-Packard via Tecan	T8+	For dispensing test compounds
Epifluorescence microscope with computer-connected digital camera	Nikon	Ti	For live cell imaging; any standard fluorescent microscope can substitute, with phase contrast or DIC optics, capable of imaging green (fluorescein), orange-red to red (Texas Red), and far-red (Cy5) fluorescence, with 100X oil objective for highest resolution
Glass-bottom tissue culture dishes	MatTek Corporation	P35G-1.5-20-C	For live cell imaging. Dishes such as the MatTek allow microscopic visualization at 600X or 1,000X magnification through use of an inverted epifluorescent or confocal microscope. These specific dishes are 3.5 cm nominal diameter, 3.3 cm inside diameter, with 20 mm diameter #1.5 thickness cover slips inserted into the bottoms.
Photoshop CS6	Adobe		Adobe photoshop or similar programs can be used to pseudocolor and merge light microscopic and fluorescent images.
Mathematica 10	Wolfram		For generation of two-dimensional isocontour isobolograms and three-dimensional surface isobolograms.