

Materials List for:

Microscopy-based Assays for High-throughput Screening of Host Factors Involved in *Brucella* Infection of Hela Cells

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Materials

Name	Company	Catalog Number	Comments
Tryptic Soy Agar (TSA)	BD	236950	
Tryptic Soy Broth (TSB)	Fluka	22092	
Kanamycin sulfate	Sigma-Aldrich	60615	
Skim milk			
250 ml screw cap bottle	Corning	8396	
DMEM	Sigma-Aldrich	D5796	
Fetal Calf Serum (FCS)	Gibco	10270	Heat-inactivated
Trypsin-EDTA (0.5%)	Gibco	15400-054	10x stock solution, dilute 1:10 in PBS
Sceptre 2.0 Cell Counter	Merck Milipore	PHCC20060	Alternative cell counting devices can be used
Greiner CELLSTAR 384-well plate	Sigma-Aldrich	M2062	
Peelable aluminum foil	Costar	6570	
Reagent dispenser: "Multidrop 384 Reagent Dispenser"	Thermo Scientific	5840150	Alternative reagent dispenser can be used.
Transfection reagent: "Lipofectamine RNAiMAX"	Invitrogen	13778-150	
Automated plate washer: "Plate washer ELx50-16"	BioTek	ELX5016	This plate washer contains a 16-channel manifold suitable for 384-well plates. It fits into a biosafety cabinet and has a lid covering the plate during washing which reduces the risk of aerosol production. Alternative plate washers with similar features could be used.
Gentamicin	Sigma-Aldrich	G1397	
Anhydrotetracycline hydrochloride	Sigma-Aldrich	37919	100 µg/ml solution in 100% ethanol is kept at -20 °C protected from light in aluminum-foil
PBS	Gibco	20012	
Paraformaldehyde	Sigma-Aldrich	P6148	Dissolve in 0.2 M HEPES buffer, pH 7.4. Store at -20 °C and thaw freshly the day before use. Caution, PFA is toxic by inhalation, in contact with skin and if swallowed.

HEPES	Sigma-Aldrich	H3375	
Triton X-100	Sigma-Aldrich	T9284	
DAPI	Roche	10236276001	
Scrambled siRNA	Dharmacon	D-001810-10	
Kif11 siRNA	Dharmacon	L-003317-00	
ARPC3 siRNA	Dharmacon	L-005284-00	
<i>Brucella abortus</i> 2308			
pJC43 (<i>apHT::GFP</i>)			Celli <i>et al.</i> ¹²
pAC042.08 (<i>apht::dsRed</i> , <i>tetO::tetR-GFP</i>)			Construction: pJC44 ⁴ was digested with EcoRI followed by generation of blunt ends with Klenov enzyme and subsequent digestion with Sall. TetR-GFP was amplified from pNF106 using primer prAC090 and prAC092. Following digestion with Sall, the TetR-GFP product was ligated to the digested pJC44 vector.
Primer prAC090	Sigma-Aldrich		TTT TTG AAT TCT GGC AAT TCC GAC GTC TAA GAA ACC
Primer prAC092	Sigma-Aldrich		TTT TTG TCG ACT TTG TCC TAC TCA GGA GAG CGT TC
HeLa CCL-2	ATCC	CCL-2	
ImageXpress Micro	Molecular Devices	IXM IMAGING MSCOPE	Automated cellular imaging microscope equipped with a precision motorized Z-stage. Alternative systems for automated microscopy and alternative components for hard- and software specified below can be employed.
High-Speed Laser Auto-Focus	Molecular Devices	1-2300-1037	
CFI Super Fluor 10X objective	Nikon	MRF00100	N.A 0.50, W.D 1.20 mm, DIC Prism: 10X, Spring loaded
Photometrics CoolSNAP HQ Monochrome CCD Camera	Molecular Devices	1-2300-1060	1,392 x 1,040 imaging pixels, 6.45 x 6.45 µm pixels, 12 bits digitization
MetaXpress software	Molecular Devices	9500-0100	
LUI-Spectra-X-7	Lumencor	SPECTRA X V-XXX-YZ	Light engine. The following light sources are used: violet (DAPI), cyan (GFP), green/yellow (RFP)
Single Band Exciter for DAPI	Semrock	FF01-377/50-25	
Single Band Emitter for DAPI	Semrock	FF02-447/60-25	
Single Band Dichroic for DAPI	Semrock	FF409-Di03-25x36	
Single Band Exciter for GFP	Semrock	FF02-472/30-25	
Single Band Emitter for GFP	Semrock	FF01-520/35-25	
Single Band Dichroic for GFP	Semrock	FF495-Di03-25x36	
Single Band Exciter for RFP	Semrock	FF01-562/40-25	
Single Band Emitter for RFP	Semrock	FF01-624/40-25	
Single Band Dichroic for RFP	Semrock	FF593-Di03-25x36	