

Materials List for:

Applying Fluorescence Resonance Energy Transfer (FRET) to Examine Effector Translocation Efficiency by *Coxiella burnetii* during siRNA Silencing

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Materials

| Name | Company | Catalog Number | Comments |
|--|-------------------------|------------------|---|
| Reagents | | | |
| Citric acid | Sigma | C0759 | ACCM-2 medium component |
| Sodium citrate | Sigma | S4641 | ACCM-2 medium component |
| Potassium phosphate | Sigma | 60218 | ACCM-2 medium component |
| Magnesium chloride | Calbiochem | 442611 | ACCM-2 medium component |
| Calcium chloride | Sigma | C5080 | ACCM-2 medium component |
| Iron sulfate | Fisher | S93248 | ACCM-2 medium component |
| Sodium chloride | Sigma | S9625 | ACCM-2 medium component |
| L-cysteine | Sigma | C6852 | ACCM-2 medium component |
| Bacto-neopeptone | BD | 211681 | ACCM-2 medium component |
| Casamino acids | Fisher | BP1424 | ACCM-2 medium component |
| Methyl beta cyclodextrin | Sigma | C4555 | ACCM-2 medium component |
| RPMI + Glutamax | ThermoFisher Scientific | 61870-036 | ACCM-2 medium component |
| Chloramphenicol | Sigma | C0378 | For bacterial culture |
| ON-TARGETplus Non-targeting Control Pool (OTP) | Dharmacon | D-001810-10-05 | Non-targeting control |
| siGENOME Human PLK1 (5347) siRNA - SMARTpool | Dharmacon | M-003290-01-005 | Causes cell death; measure of transfection efficiency |
| siGENOME Human RAB5A (5968) siRNA - SMARTpool | Dharmacon | M-004009-00-0005 | |
| siGENOME Human RAB7A (7879) siRNA - SMARTpool | Dharmacon | M-010388-00-0005 | |
| 5x siRNA buffer | Dharmacon | B-002000-UB-100 | Use sterile RNase-free water to dilute 5x siRNA buffer to 1x siRNA buffer |
| DharmaFECT-1 Transfection Reagent | Dharmacon | T-2001-01 | For transfection |
| Opti-MEM + GlutaMAX | ThermoFisher Scientific | 51985-034 | Reduced-serum medium used for transfection |
| DMEM + GlutaMAX | ThermoFisher Scientific | 10567-014 | For cell culture and infection |
| Heat-inactivated fetal calf serum (FCS) | Thermo Scientific | SH30071.03 | Can use alternate equivalent product |
| DMSO | Sigma | D8418 | For storage of <i>Coxiella</i> strain |
| PBS | | | For cell culture |
| 0.05% Trypsin + EDTA | ThermoFisher Scientific | 25300-054 | For cell culture |
| dH ₂ O | | | For dilution of samples and standards for qPCR |

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|---|-------------------------|-----------------------|--|
| Quick-gDNA Mini Prep | ZYMO Research | D3007 | Can use alternate equivalent product to extract gDNA |
| SensiFAST SYBR No-ROX Kit | Bioline | BIO-98020 | Can use alternate equivalent qPCR master mix product for qPCR reaction |
| LiveBLAzer FRET-B/G Loading kit with CCF2-AM | Invitrogen | K1032 | For measurement of translocation using fluorescence and generation of the 6X loading solution (contains Solution A, B, C and DMSO) |
| Sodium hydroxide | Merck | 106469 | Probenicid solution component |
| Sodium phosphate monobasic | Sigma | 71505 | Probenicid solution component |
| di-Sodium hydrogen orthophosphate | Merck | 106586 | Probenicid solution component |
| Probenicid | Sigma | P8761 | Probenicid solution component |
| DRAQ5 Fluorescent Probe Solution (5 mM) | ThermoFisher Scientific | 62251 | Nuclei stain to determine cell viability. Use 1:4000 diluted in PBS. |
| 4% PFA (paraformaldehyde) solution in PBS | Sigma | P6148 | Fixing solution for HeLa 229 cells |
| 25 cm ² tissue culture flask with vented cap | Corning | 430639 | For growth of bacterial strain |
| 96 Well Flat Clear bottom Black Polystyrene TC-Treated Microplates, Individually wrapped, with Lid, Sterile | Corning | 3603 | |
| 75 cm ² tissue culture flask with vented cap | Corning | 430641 | For growth of HeLa 229 cells |
| 175 cm ² tissue culture flask with vented cap | Corning | 431080 | For growth of HeLa 229 cells |
| Haemocytometer | | | For quantification of HeLa 229 cells |
| Tear-A-Way 96 Well PCR plates | 4titude | 4ti-0750/TA | For qPCR reaction |
| 8-Lid chain, flat | Sarstedt | 65.989.002 | For qPCR reaction |
| Name | Company | Catalog Number | Comments |
| Equipment | | | |
| Bench top microfuge | | | |
| Bench top vortex | | | |
| Orbital mixer | | | |
| Centrifuge | Eppendorf | 5810 R | For pelleting bacterial culture |
| Nanodrop | | | For gDNA quantification |
| Mx3005P QPCR machine | Aligent Technologies | 401456 | For quantification of Coxiella genomes in 7 day culture |
| ClarioSTAR microplate reader | BMG LabTech | | For measurement of fluorescence |