

Materials List for

Real-Time Quantitative Measurement of Tumor Cell Migration and Invasion Following Synthetic mRNA Transfection

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Materials

| Name | Company | Catalog Number | Comments |
|---|---------------------------|----------------|--|
| Alphamager HP | ProteinSimple | 92-13823-00 | Agarose gel imaging system |
| α -Tubulin antibody | Sigma | T9026 | Used to detect α -tubulin protein (dilution 1:3,000) |
| CIM-plate 16 | Agilent Technologies, Inc | 5665825001 | Cell invasion and migration plates |
| Crk antibody | BD Biosciences | 610035 | Used to detect CrkI and CrkII proteins (dilution 1:1,500) |
| CrkL antibody | Santa Cruz | sc-319 | Used to detect CrkL protein (dilution 1:1,500) |
| Dulbecco's Modified Eagle's Medium (DMEM) | ATCC | 302002 | Cell culture medium |
| Dulbecco's phosphate-buffered saline (DPBS) | Corning | 21-031-CV | Buffer used to wash cells |
| Fetal bovine serum (FBS) | Hyclone | SH30910.03 | Culture medium supplement |
| Heracell VIOS 160i CO2 incubator | Thermo Scientific | 51030285 | CO2 incubator |
| IRDye 800CW goat anti-mouse IgG secondary antibody | Li-Cor | 926-32210 | Secondary antibody for Western blot analysis (dilution 1:10,000) |
| IRDye 800CW goat anti-rabbit IgG secondary antibody | Li-Cor | 926-32211 | Secondary antibody for Western blot analysis (dilution 1:10,000) |
| Lithium chloride | Invitrogen | AM9480 | Used for RNA precipitation |
| Matrigel matrix | Corning | 354234 | Extracellular matrix (ECM) gel |
| MEGAscript T7 transcription kit | Invitrogen | AM1334 | Used for RNA synthesis |
| Millennium RNA markers | Invitrogen | AM7150 | Used for formaldehyde agarose gel electrophoresis |
| Mini centrifuge | ISC BioExpress | C1301P-ISC | Used to spin down cells |
| Mouse brain QUICK-Clone cDNA | TaKaRa | 637301 | Source of genes (inserts) for cloning |
| NanoQuant | Tecan | M200PRO | Nucleic acid quantification system |
| Neon electroporation system | ThermoFisher Scientific | MPK5000 | Electroporation system ¹ |
| Neon transfection system 10 μ L kit | ThermoFisher Scientific | MPK1025 | Electroporation kit |

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|--|---------------------------|------------|--|
| Neon transfection system 100 µL kit | ThermoFisher Scientific | MPK10096 | Electroporation kit |
| NorthernMax denaturing gel buffer | Invitrogen | AM8676 | Used for formaldehyde agarose gel electrophoresis |
| NorthernMax formaldehyde load dye | Invitrogen | AM8552 | Used for formaldehyde agarose gel electrophoresis |
| NorthernMax running buffer | Invitrogen | AM8671 | Used for formaldehyde agarose gel electrophoresis |
| Nuclease-free water | Teknova | W3331 | Used for various reactions during mRNA synthesis |
| Odyssey CLx Imager | Li-Cor | | Imager for Western blot analysis |
| pcDNA3.1/myc-His | Invitrogen | V80020 | The vector into which inserts (mouse Crkl and CrkL cDNAs) were cloned |
| pFLAG-CMV-5a | Millipore Sigma | E7523 | Source of the FLAG epitope tag |
| Phenol:chloroform:isoamyl alcohol | Sigma | P2069 | Used for DNA extraction |
| PmeI | New England BioLabs | R0560L | Used to linearize the plasmids for mRNA synthesis |
| Poly(A) tailing kit | Invitrogen | AM1350 | Used for poly(A) tail reaction |
| Polystyrene tissue culture dish (100 x 20 mm style) | Corning | 353003 | Used for culturing cells before transfection |
| Polystyrene tissue culture dish (35 x 10 mm style) | Corning | 353001 | Used for culturing transfected cells |
| Proteinase K | Invitrogen | 25530049 | Used to remove protein in the reaction mixture |
| Purifier Axiom Class II, Type C1 | Labconco Corporation | 304410001 | Biosafety cabinet for sterile handling of cells |
| Resuspension Buffer R | ThermoFisher Scientific | | A buffer included in the electroporation kits, MPK1025 and MPK10096. The buffer is used to resuspend cells before electroporation, and its composition is proprietary information. |
| RNaseZap | Invitrogen | AM9780 | RNA decontamination solution |
| Scepter | Millipore | C85360 | Handheld automated cell counter |
| ScriptCap 2'-O-methyltransferase kit | Cellscript | C-SCMT0625 | Used for capping reaction |
| ScriptCap m ⁷ G capping system | Cellscript | C-SCCE0625 | Used for capping reaction |
| Sodium dodecyl sulfate solution | Invitrogen | 15553-035 | Detergent used for the proteinase K reaction |
| Sorvall Legend XT centrifuge | Thermo Scientific | 75004532 | Benchtop centrifuge to spin down cells |
| Trypsin-EDTA | Gibco | 25300-054 | Used for dissociation of cells |
| U-118MG | ATCC | HTB15 | An adherent cell line derived from a human glioblastoma patient |
| Vinculin antibody | Sigma | V9131 | Used to detect vinculin protein (dilution 1:100,000) |
| xCELLigence RTCA DP | Agilent Technologies, Inc | 380601050 | Instrument used for real-time cell analysis |
| ¹ Electroporation parameters and other related information for various cell lines are available on the manufacturer's homepage (https://www.thermofisher.com/us/en/home/life-science/cell-culture/transfection/neon-transfection-system/neon-transfection-system-cell-line-data.html?). | | | |