

# Construction of Cyclic Cell-Penetrating Peptides for Enhanced Penetration of Biological Barriers

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## Materials

| Name                                                                           | Company         | Catalog Number  | Comments                                                                                                                                   |
|--------------------------------------------------------------------------------|-----------------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| 1,2-ethanedithiol                                                              | Aladdin         | K1722093        | stench                                                                                                                                     |
| 2-(7-Azobenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) | HEOWNS          | A-0443697       |                                                                                                                                            |
| 4,4'-bis(bromomethyl)biphenyl                                                  | TCI             | B1921           |                                                                                                                                            |
| 4T1 cells                                                                      | ATCC            |                 | 4T1 cells were cultured in DMEM medium supplemented with 10% FBS (Hyclone) in a 37 °C humidified incubator containing 5% CO <sub>2</sub> . |
| Acetonitrile                                                                   | Adamas          | 1484971         | toxicity                                                                                                                                   |
| Dichloromethane                                                                | Energy          | W330229         | skin harmful                                                                                                                               |
| Diethyl ether                                                                  | Aldrich         | 673811          | flammable                                                                                                                                  |
| Dimethyl sulfoxide                                                             | Beyotime        | ST038           | skin harmful                                                                                                                               |
| Dulbecco's Modified Eagle Medium (DMEM)                                        | Gibco           |                 |                                                                                                                                            |
| Electrospray Ionization Mass Spectrometer                                      | Waters          | G2-S Tof        |                                                                                                                                            |
| Ethylene Diamine Tetraacetic Acid (EDTA)                                       | BioFroxx        | 1340            |                                                                                                                                            |
| Fetal bovine serum (FBS)                                                       | HyClone         |                 |                                                                                                                                            |
| Flow cytometer                                                                 | Beckman Coulter | CytoFLEX        |                                                                                                                                            |
| Fluorescein isothiocyanate isomer (FITC)                                       | Energy          | E0801812500     |                                                                                                                                            |
| Fluorescent microscope                                                         | Carl Zeiss      | Axio Observer 7 |                                                                                                                                            |
| Fmoc-Arg(Pbf)-OH                                                               | HEOWNS          | F-81070         |                                                                                                                                            |
| Fmoc-Cys(Trt)-OH                                                               | GL Biochem      | GLS201115-35202 |                                                                                                                                            |
| Fmoc-βAla-OH                                                                   | Adamas          | 51341C          |                                                                                                                                            |
| HeLa cells                                                                     | ATCC            |                 | HeLa cells were cultured in DMEM supplemented with 10% FBS (Hyclone) in a 37 °C humidified incubator containing 5% CO <sub>2</sub> .       |

|                                               |                    |                                                                          |                                      |
|-----------------------------------------------|--------------------|--------------------------------------------------------------------------|--------------------------------------|
| High-Performance Liquid Chromatography        | Agilent            | Agilent 1260                                                             |                                      |
| High-Performance Liquid Chromatography column | Agilent            | Poroshell EC-C18 120, 4.6 × 150 mm (pore size 120 Å, particle size 4 µm) |                                      |
| Lyophilizer                                   | SP Scientific      | Vir Tis                                                                  |                                      |
| Methanol                                      | Aldrich            | 9758                                                                     | toxicity                             |
| Microtiter plate                              | Thermo µdrop plate | N12391                                                                   |                                      |
| Morpholine                                    | HEOWNS             | M99040                                                                   | irritant                             |
| Multi-technology microplate reader            | Thermo             | VARIOSKAN LUX                                                            |                                      |
| N,N-Diisopropylethylamine                     | HEOWNS             | E-81416                                                                  | irritant                             |
| N,N-Dimethyl formamide                        | Energy             | B020051                                                                  | harmful to skin                      |
| Poly-Prep column                              | Bio-Rad            | 7321010                                                                  | polypropylene chromatography columns |
| Rink Amide MBHA resin (0.572 mmol/g)          | GL Biochem         | GLS180301-49101                                                          |                                      |
| Three-way stopcocks                           | Bio-Rad            | 7328107                                                                  |                                      |
| Tissue culture plate insert                   | LABSELECT          | 14211                                                                    |                                      |
| Trifluoroacetic acid                          | HEOWNS             | T63278                                                                   | corrosive                            |
| Triisopropylsilane                            | HEOWNS             | T-0284475                                                                |                                      |
| Trypsin                                       | BioFroxx           | 1004                                                                     |                                      |
| Vacuum manifold                               | Promega            | A7231                                                                    |                                      |