

CellPore™ MECHANOPORATION GUIDE

Optimized Parameters for Efficient Intracellular Delivery

Use this guide to help you optimize intracellular delivery with the CellPore™ Transfection System. This resource provides the essential operating parameters required for successfully performing mechanoporation across a wide range of cell types. To streamline your workflow, the guide is organized into two sections:

Section 1: Validated Cell Types - Refer here first to see if your cell type has been empirically tested by our scientists. For these cells, we provide optimal parameters, including the recommended CellPore™ Delivery Cartridge, Delivery Medium, delivery pressure ranges, and cell numbers.

Section 2: Unvalidated Cell Types - If your specific cell type is not listed in Section 1 (Table 1), use this section to find a framework for selecting the ideal cartridge size based on cell diameter, alongside baseline parameters to start your optimization.

Section 1: Guidelines for Validated Cell Types

Refer to Table 1 below for a list of current validated cell types and their optimized parameters for mechanoporation using CellPore™ .

Table 1. Optimized CellPore™ Parameters for Validated Cell Types

| Cell Type | Delivery Cartridge | Delivery Medium | Pressure Range | Run Time (s) | Cell Number/ Range | |
|--------------|--|-----------------|----------------|--------------|--------------------|--|
| Immune Cells | T cell (human), unactivated | 300 | A | 50 - 90 psi | 3 | 5 x 10 ⁵ to 2.5 x 10 ⁷ |
| | T cell (mouse), unactivated | 300 | A | 30 - 70 psi | 3 | 2 x 10 ⁶ |
| | NK cell (human), unactivated | 300 | A | 50 - 90 psi | 3 | 5 x 10 ⁵ to 2 x 10 ⁷ |
| | B cell (human), unactivated | 300 | A | 40 - 80 psi | 3 | 2 x 10 ⁶ |
| | Monocyte (human) | 300 | A | 20 - 50 psi | 5 | 1 x 10 ⁶ |
| | PBMC (human) | 300 | A | 30 - 90 psi | 5 | 5 x 10 ⁵ to 5 x 10 ⁶ |
| Stem Cells | CD34+ HSPC (human), umbilical cord blood | 300 | A | 15 - 60 psi | 10 | 1 x 10 ⁴ to 5 x 10 ⁵ |
| | CD34+ HSPC (human), mobilized PB | 300 | A | 15 - 60 psi | 10 | 2 x 10 ⁴ to 1 x 10 ⁶ |
| | Induced Pluripotent Stem Cell (human) | 500 | B | 10 - 30 psi | 5 | 3 x 10 ⁵ to 2 x 10 ⁶ |
| | Embryonic Stem Cell (human), H9 | 500 | B | 10 - 30 psi | 5 | 3 x 10 ⁵ to 2 x 10 ⁶ |
| Cell Lines | Jurkat | 300 | A | 30 - 50 psi | 3 | 1 x 10 ⁶ |
| | HEK293 (suspension) | 500 | A | 5 - 25 psi | 3 | 1 x 10 ⁶ |

Operational Guidelines:

Reaction Volume: A standard 50 μL reaction volume is recommended across all validated cell types. In instances where cell recovery is low, the reaction volume may be increased to 100 μL .

Cell Number Range: When a single value is listed for cell numbers, it indicates the established baseline used during testing. This value is not the absolute minimum or maximum capacity, and users will likely be able to scale up or down. Conversely, when a range of cell numbers is provided, it represents the known minimum and maximum capacities for that specific cell type.

Sample Preparation: Cells must be fully dissociated into a single-cell suspension. Cell clumping will disrupt flow through the cartridge and result in inconsistent mechanoporation.

Cartridge Preparation & Handling: Pre-load the CellPore™ Delivery Cartridge collection tube with culture medium to enhance post-delivery viability and recovery. Once the instrument completes the run, briefly centrifuge the collection tube to ensure complete volume recovery.

Refer to Table 2 below for a list of cargo types and recommended starting quantities or concentrations for mechanoporation.

Table 2. Recommended Quantities of Cargoes for Mechanoporation

| Cargo | Cell Type |
|------------------------|--|
| Fluorescent Dextran | 0.1 mg/mL |
| mRNA, saRNA | 10 - 15 pmol (e.g. 5 μg for a 1kb mRNA construct) |
| Cas9 Ribonucleoprotein | 60 - 80 pmol (1:2.5 Cas9:gRNA) glycerol-free Cas9 nuclease highly recommended |
| Plasmid DNA | 2 - 10 μg (for ~3.5 kb) |

Note: Optimal concentrations and overall outcomes may differ depending on the specific cell type being used. Other types of cargo beyond those listed may also be suitable.

Section 2: Guidelines for Unvalidated Cell Types

For successful mechanoporation, it is essential to choose the correct CellPore™ Delivery Cartridge size by matching it to the diameter of your cell type. Selecting a cartridge that is too small can lead to low cell viability (and clogging), while one that is too large may result in poor transfection efficiency.

To start your optimization, measure the average diameter of your cells in suspension. This measurement will guide you in selecting the appropriate CellPore™ Delivery Cartridge and corresponding CellPore™ Delivery Medium for your experiment (refer to Table 3). Generally, we recommend beginning with Delivery Medium A for most cell types.

Table 3. General CellPore™ Parameters Based on Cell Diameter

| Cell Diameter | CellPore™ Delivery Cartridge | CellPore™ Delivery Medium |
|-----------------------|------------------------------|---------------------------|
| 6 - 9 μm | 300 | A |
| 8 - 10 μm | 400 | A |
| 10 - 13 μm | 500 | A |
| 13 - 15 μm | 600 | A |
| 15 - 18 μm | 700 | A |

Note: These guidelines provide a starting reference. However, additional cell type properties such as membrane stiffness can also influence cartridge compatibility. It is recommended to test a few cartridge sizes in order to determine best performance.





To optimize CellPore™ parameters for your application, see the protocol on [Optimizing Delivery Efficiency with Fluorescent Dextran](#). Scan the QR code to view this protocol. Refer to Table 4, below for recommended starting parameters and Table 2 for cargo recommendations.



View Protocol

www.stemcell.com/CellPore-protocol

Table 4. Recommended Starting Parameters for Optimization

| Parameter | Value |
|--|---|
|  Delivery Pressure | Broad Optimization: 20 - 100 psi Sensitive Cell Type: 15 - 60 psi Robust Cell Type: 50 - 90 psi |
|  Run Time | 5 - 10 sec (or run manually) |
|  Cell Number | Starting Range: 1×10^5 - 1×10^6 |
|  Reaction Volume | 50 μ L |



For more information about the CellPore™ Transfection System, please visit CellPore.com or contact your local STEMCELL representative.

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