# CellPore™ Transfection Kit 300

**For processing 12 samples with CellPore™ Transfection System**

Catalog #100-1020 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Product Description

CellPore™ Transfection Kit 300 comprises reagents and consumables that have been optimized for direct cytosolic delivery of a range of molecules (including nucleic acids, proteins, small molecules, and combinations thereof) to compatible cell types on CellPore™ Transfection System (Catalog #100-0946). These include unactivated immune cells, peripheral blood mononuclear cells (PBMCs), and CD34+ hematopoietic stem and progenitor cells (HSPCs). The kit includes a positive delivery control (FITC-Dextran) that enables same-day optimization of delivery efficiency.

## Product Information

The following components are sold as part of CellPore™ Transfection Kit 300 (Catalog #100-1020) and are not available for individual sale.

| COMPONENT NAME | COMPONENT # | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| CellPore™ Delivery Medium | 100-1023 | 2 x 2.5 mL | Store at 2 - 8°C.<br>Do not freeze. | Stable until expiry date (EXP) on label. |
| CellPore™ FITC-Dextran | 100-1024 | 0.15 mL | Store at -20°C. | Stable until expiry date (EXP) on label. |
| CellPore™ Delivery Cartridges 300 | 100-0998 | 12 Cartridges/Bag | Store at 15 - 25°C. | Stable for 5 years from date of manufacture (MFG) on label. |

## Preparation of Reagents and Materials

A. CELL ISOLATION

Use of standardized cell isolation and/or culture methods are important considerations to obtain the best results using CellPore™ Transfection System. We recommend using EasySep™ cell isolation kits to obtain high quality, viable cells from fresh leukapheresis or whole blood samples. Refer to Table 1 or visit www.stemcell.com/easysep for available cell isolation kits.

**Table 1. List of Recommended Cell Isolation Products**

| CELL TYPE | PRODUCT NAME | CATALOG # |
|---|---|---|
| T cells | EasySep™ Human T Cell Isolation Kit | 17951 |
| Natural killer (NK) cells | EasySep™ Human NK Cell Isolation Kit | 17955 |
| CD34+ HSPCs | EasySep™ Human Progenitor Cell Enrichment Kit II<br>OR<br>EasySep™ Human Cord Blood CD34 Positive Selection Kit II | 17936<br>OR<br>17896 |
| PBMCs | Lymphoprep™<br>AND<br>SepMate™-50 (IVD) | 07851<br>AND<br>85450 |

Alternatively, see www.stemcell.com/primarycells for information on ready-to-use, fresh, and frozen cell samples.

NOTE: Cell viability may be improved by resting cells in culture medium in a humidified incubator with 5% $CO_2$ at 37°C for a minimum of 30 minutes prior to handling on CellPore™ Transfection System. Optionally, certain cell types may be rested overnight and used the next day.

B. CELL PREPARATION

The following example is for preparing a T cell master mix for seven 20 µL reactions (2 x 10^6 cells/reaction). The volumes and cell numbers indicated below refer to the exact amount required for the pressure sweep experiment. A small excess should be included in the preparation of these samples to account for pipetting variations. If preparing other cell types, refer to Table 2 and adjust accordingly.

1. Transfer 1.4 x10^7 T cells to a new tube and centrifuge at 500 x *g* for 5 - 10 minutes at room temperature (15 - 25°C).
2. Remove supernatant completely and resuspend cell pellet in 140 µL of CellPore™ Delivery Medium. Gently pipette the cells to obtain a single-cell suspension. The resulting T cell concentration should be 1 x 10^8 cells/mL. Immediately proceed to Directions for Use.

**Table 2. Recommended number of cells per reaction**

| CELL TYPE | NUMBER OF CELLS PER REACTION |
|---|---|
| Isolated T cells | 5 x 10^5 - 2.5 x 10^7 |
| Isolated NK cells | 5 x 10^5 - 2 x 10^7 |
| CD34+ HSPCs | 5 x 10^4 - 2 x 10^6 |
| PBMCs | < 5 x 10^6 |

## Directions for Use

PLEASE READ THE ENTIRE PROTOCOL BEFORE PROCEEDING.

The following protocol describes the general guidelines for conducting a pressure sweep for optimizing the delivery of CellPore™ FITC-Dextran to primary cells using the CellPore™ Transfection Kit 300. Depending on the experimental objective, the FITC-Dextran cargo can either be substituted or co-delivered with a different target molecule.

The experiment detailed below comprises a set of five reactions plus two controls. The CellPore™ delivery pressure that provides the highest delivery efficiency with lowest impact to viability can be selected for subsequent experiments.

In some cases, a subsequent pressure sweep and/or other biological parameter optimizations may be required to be performed by using the remaining cartridges in order to fine-tune the optimal delivery and cell viability conditions. It is highly recommended to confirm and/or fine-tune the optimal delivery pressure with the cargo of interest.

For complete instructions on using CellPore™ Transfection System, refer to the CellPore™ User Reference Manual (Document #10000018433), available at www.stemcell.com, or contact us to request a copy.

A. CELLPORE™ PRESSURE SWEEP

Best results are obtained when limiting prolonged cell exposure to ambient temperature conditions. It is therefore important to work as quickly as possible and consider keeping unused cells in a humidified incubator with 5% $CO_2$ at 37°C when performing larger experiments.

1. Set aside a 20 µL aliquot of the cell suspension (see Preparation, section B) at room temperature. This represents the 'Untreated' condition.
2. Add 6 µL of CellPore™ FITC-Dextran to the remaining 120 µL cell suspension to achieve a final FITC-Dextran concentration of 0.1 mg/mL. Gently mix by pipetting.

   NOTE: Optionally, an alternative cargo may be added in lieu or in addition to FITC-Dextran during this step. For best results, the total amount of cargo addition should not exceed 10% of the reaction volume. Generally, a final cargo concentration of 0.1 mg/mL per reaction represents a good starting point for optimization.
3. Transfer 20 µL of the cell suspension to a new CellPore™ Delivery Cartridge 300. Always insert the pipette tip to the bottom of the cartridge when dispensing the sample (Figure 1).

   NOTE: Do not centrifuge the cartridge at this stage as this will lead to a loss in delivery performance. Gently tap the cartridge several times to collect volume at the bottom if necessary.

**Figure 1. Proper Pipetting Technique for CellPore™ Delivery Cartridge 300**

4. Close the top lid and ensure the cartridge insert is securely placed in the collection tube.
5. Place the cartridge into the arm of the CellPore™ Transfection System.
6. Set instrument pressure to the appropriate pressure (e.g. 30 psi) and run the sample.
7. Retrieve the cartridge from the instrument once completed. The cell sample should be at the bottom or side of the collection tube.

   NOTE: For smaller reaction volumes, it is recommended to spin down the cartridge in a mini centrifuge for a few seconds for full volume recovery.
8. Remove and dispose of the cartridge insert. Immediately add 180 µL of CellPore™ Delivery Medium to the collection tube and gently mix to resuspend cells. Cap the lid and set aside at room temperature.
9. Repeat steps 3 - 8 for all remaining pressure conditions in order to complete the series: 30, 50, 70, 90, and 110 psi.
10. Once all pressure conditions are complete, set aside a 20 µL aliquot of the prepared cell suspension (i.e. cells + cargo). This represents the 'Endocytosis' control for measuring the natural cell uptake efficiency of the target molecule.
11. Add 180 µL of CellPore™ Delivery Medium to both the 'Untreated' and 'Endocytosis' control aliquots from steps 1 and 10.
12. Transfer reactions to a 96-well plate and incubate in a humidified incubator with 5% $CO_2$ at 37°C for 2 hours. Proceed to section B for downstream analysis.

    NOTE: Optionally, exchange cells from all reactions into an appropriate culture medium and transfer to a 96-well plate for incubation in a humidified incubator with 5% $CO_2$ at 37°C until ready for analysis.

B. DOWNSTREAM ANALYSIS

1. Measure cell viability and delivery efficiency by flow cytometry. It is recommended to conduct flow cytometry analysis on the same day of the experiment for measuring FITC-Dextran delivery.

   NOTE: Stain cells with appropriate cell surface markers to facilitate analysis. It is also recommended to include a viability dye.
2. If desired, plate remaining cells in an appropriate culture system for further downstream analysis. In this case, it is recommended to wash the cells once in culture medium before plating.

## Notes and Tips

- Cells isolated by EasySep™ positive selection may show decreased cell viability when manipulated on the CellPore™ Transfection System.
- Cells isolated from older PBMCs or leukapheresis samples (> 48 hours post draw) may result in lower viability for some donors.
- When using cryopreserved cells, care must be taken to fully wash away cryoprotectants (e.g. dimethyl sulfoxide [DMSO]) from the cell suspension. Cell viability may be improved by resting cells in culture medium in a humidified incubator with 5% $CO_2$ at 37°C for a minimum of 60 minutes prior to handling on CellPore™ Transfection System.
- Best results are obtained when limiting prolonged cell exposure to ambient temperature conditions. Consider keeping unused cells in a humidified incubator with 5% $CO_2$ at 37°C when performing larger experiments.
- Undelivered cargo may lead to lower viability in certain cell culture systems. It is therefore recommended to wash the cells with an appropriate culture medium prior to plating for downstream culture.
- Isolated or cargo-delivered cells may be cryopreserved using CryoStor® CS5 (Catalog #07933) or CryoStor® CS10 (Catalog #07930) and stored at -135°C for future use.

ASSESSING VIABILITY AND DELIVERY EFFICIENCY

For flow cytometry analysis, FITC-Dextran can be assessed on the blue laser (FITC channel). If compensation is required, consider using an aliquot of a delivered cell sample.

The following fluorochrome-conjugated antibodies and dyes are recommended in order to facilitate analysis of your cell type of interest:

- Anti-Human CD45 Antibody, Clone HI30 (Catalog # 60018)
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011)
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013) or Clone 8G12 (Catalog #60121)
- Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021)
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004)
- Viability Dye, including 7-AAD (7-Aminoactinomycin D; Catalog #75001) or Propidium Iodide (Catalog #75002)

# Data

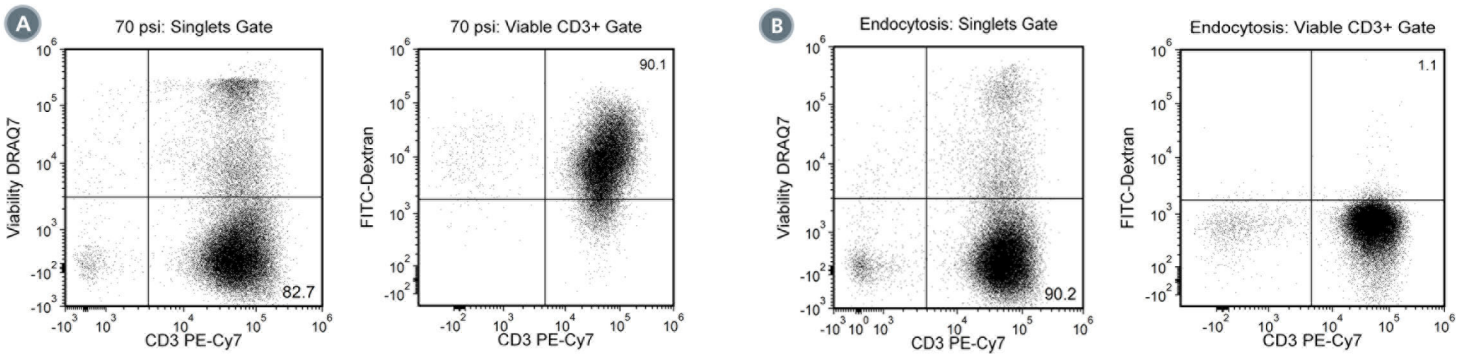


**Figure 2.** Starting from T cells isolated using EasySep™ Human T Cell Isolation Kit, the delivery efficiency of CellPore™ FITC-Dextran **(A)** at 70 psi is typically 92.0 ± 3.3% with a typical viability drop of 8.2 ± 6.6% (mean ± SD). **(B)** Under unmanipulated conditions, the natural uptake of FITC-Dextran by T cells (i.e. endocytosis) is typically below 1% (n = 18). In the above example, the input and output T cell viability was 90.2% and 82.7% respectively, and FITC-Dextran delivery efficiency was 90.0%.

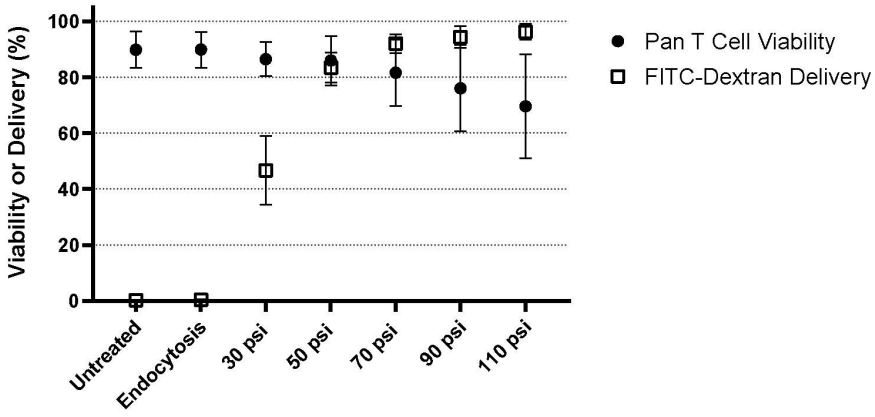


**Figure 3.** Representative pressure sweep graph for identification of the optimal delivery parameter (i.e. system pressure). CellPore™ FITC-Dextran was delivered to freshly isolated human T cells under increasing instrument pressure. Both cell viability and FITC-Dextran delivery efficiency (i.e. FITC+CD3+ cells) were measured by flow cytometry on the same day of the experiment. Input cell viability and natural cellular uptake of FITC-Dextran are shown by both untreated and endocytosis control conditions. Optimal delivery can be identified by the condition that measures a saturation of the delivery efficiency with limited impact to cell viability. Further fine-tuning of the optimal delivery pressure can be performed in a subsequent experiment. Data are shown as mean ± SD, n = 18.