CellPore™ Transfection Kit 300

For processing 12 samples with CellPore™ Transfection System

Catalog #100-1020 1 Kit

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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. 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	Store at 15 - 25°C.	Stable for 5 years from date of manufacture (MFG) on label.

Preparation of Reagents and Materials

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 for Compatible Cell Types

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 OR EasySep™ Human Cord Blood CD34 Positive Selection Kit II	17936 OR 17896
PBMCs	Lymphoprep™ AND SepMate™-50 (IVD) OR SepMate™-15 (IVD)	07851 AND 85450 OR 85415

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 1 hour prior to handling on CellPore™ Transfection System. Optimal resting and/or pre-culture periods will need to be determined and will vary depending on the source and cell type.

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 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 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 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. CELL PREPARATION

The following protocol is for preparing a master mix for 7 x 50 µL reactions to perform a five-point pressure sweep optimization experiment, including two controls. The recommended number of cells for each compatible cell type are provided in Table 2. For additional details regarding compatible cell number ranges, refer to Table 4.

Table 2. Number of Cells Required for 7 x 50 µL Reactions (350 µL Total) for Compatible Cell Types

CELL TYPE	NUMBER OF CELLS PER REACTION	TOTAL NUMBER OF CELLS REQUIRED	CELL CONCENTRATION (cells/mL)
Isolated T cells	2 x 10^6	1.4 x 10^7	4 x 10^7
Isolated NK cells	1 x 10^6	7 x 10^6	2 x 10^7
CD34+ HSPCs	1 x 10^5	7 x 10^5	2 x 10^6
PBMCs	2.5 x 10^6	1.75 x 10^7	5 x 10^7

The following example is for preparing a T cell master mix for 7 x 50 µL reactions (2 x 10^6 T cells/reaction). The volumes and cell numbers refer to the exact amounts required; however, a small excess should be included in the preparation of these samples to account for pipetting error. If using other cell types, adjust cell numbers accordingly.

1. Transfer 1.4 x10^7 isolated T cells to a new tube and centrifuge at 500 x g for 5 - 10 minutes at room temperature (15 - 25°C).
2. Remove the supernatant and resuspend the cell pellet in 350 µL of CellPore™ Delivery Medium. Gently pipette the cells to obtain a single cell suspension. The resulting T cell concentration should be 4 x 10^7 cells/mL. Immediately proceed to section B.

B. CELLPORE™ PRESSURE SWEEP

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

1. Thaw CellPore™ FITC-Dextran at room temperature (15 - 25°C). Vortex to mix.

   NOTE: If not used immediately, store CellPore™ FITC-Dextran at 2 - 8°C for up to 3 months, or aliquot and store at -20°C. Protect from light. Do not exceed the expiry date as indicated on the label. Frozen aliquots may be thawed up to 5 times without diminished performance.
2. Program the CellPore™ Transfection System to the recommended pressures and run times according to Table 3.

   Table 3. Recommended Test Pressures and Run Times for Compatible Cell Types

CELL TYPE	RECOMMENDED PRESSURE SWEEP (PSI)	RUN TIME (seconds)
Isolated T cells	30, 50, 70, 90, 110	3
Isolated NK cells	30, 50, 70, 90, 110	3
CD34+ HSPCs	10, 15, 20, 25, 30	10
PBMCs	20, 40, 60, 80, 100	5

3. Set aside a 50 µL aliquot of the cell suspension (prepared in section A step 2) at room temperature. This represents the 'Untreated' condition.
4. Add 15 µL of CellPore™ FITC-Dextran to the remaining 300 µL of the cell suspension to achieve a final concentration of 0.1 mg/mL. Gently mix by pipetting.

   NOTE: Optionally, an alternative cargo may be added in lieu or in addition to CellPore™ FITC-Dextran during this step. For best results, the total volume of cargo added 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.

5. Transfer 50 µL of the cell suspension into a new CellPore™ Delivery Cartridge 300. Always insert the pipette tip to the bottom of the Cartridge Insert when dispensing the sample (Figure 1) and avoid creating bubbles.

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

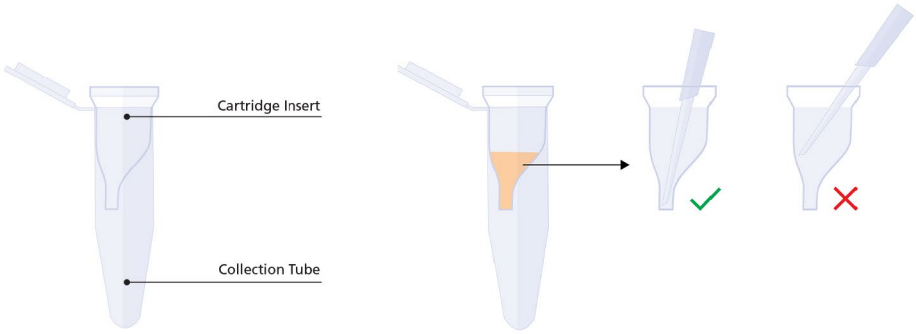

Figure 1. Proper Pipetting Technique for CellPore™ Delivery Cartridge

6. Close the cap and ensure the Cartridge Insert is securely placed in the Collection Tube.
7. Place the Delivery Cartridge into the Cartridge Holder of the CellPore™ Transfection System.
8. Run the first sample and 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.
9. Remove and dispose of the Cartridge Insert.
10. Immediately add 150 µL of CellPore™ Delivery Medium to the Collection Tube and gently mix to resuspend cells. Close the cap and set aside at room temperature.

    NOTE: If delivering alternative cargos that require a cell culture step prior to analysis, refer to Notes and Tips for details on pre-loading the Collection Tube with culture medium and resting steps for optimal results.
11. Repeat steps 5 - 10 for all remaining pressure conditions in the recommended pressure sweep (as shown in Table 3).
12. Once all pressure conditions are complete, set aside a 50 µL aliquot of the cell suspension (i.e. cells + cargo) prepared in step 4. This represents the 'Endocytosis' control for measuring the natural cellular uptake of the target molecule.
13. Add 150 µL of CellPore™ Delivery Medium to both the 'Untreated' and 'Endocytosis' control aliquots from steps 3 and 12, respectively.
14. Incubate at 37°C and 5% CO_2 for a minimum of 30 minutes up to 2 hours.

Proceed to section C for downstream analysis via flow cytometry.

C. DOWNSTREAM ANALYSIS

Cell viability and FITC-dextran delivery efficiency may be assessed by flow cytometry, preferably on the same day of the experiment. FITC-dextran can be measured 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 for analysis of the 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)
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)
- Viability Dye, including 7-AAD (7-Aminoactinomycin D; Catalog #75001) or Propidium Iodide (Catalog #75002)

Notes and Tips

- For delivering cargo that requires a cell culture step prior to analysis, it is recommended to pre-load the Collection Tube of the CellPore™ Delivery Cartrdige (Figure 1) as follows:
  1. Remove the Cartridge Insert and add 150 µL of an appropriate cell culture medium warmed to 37°C to the Collection Tube.
  2. Re-insert the Cartridge Insert into the Collection Tube and proceed as directed in section B (step 5 onward). Omit the addition of CellPore™ Delivery Medium to the Collection Tube (i.e. section B step 10).
  3. Discard the Cartridge Insert and cap the Collection Tube. Incubate at 37°C and 5% CO_2 for 2 hours prior to transferring to a culture plate or mixing in order to safeguard cell viability. This resting period may need to be optimized depending on the cell type.
- CellPore™ Delivery Cartridge is compatible with cells isolated with EasySep™ positive and negative isolation kits.
- 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 at least 1 hour prior to handling on the CellPore™ Transfection System.
- 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.
- The reaction volume range for the CellPore™ Delivery Cartridge 300 is 20 - 200 µL. The recommended minimum reaction volume is 50 µL, as smaller volumes may lead to suboptimal results.
- In case of low cell recovery, consider increasing the reaction volume. In this case, keep the cell number and cargo concentration constant.

Table 4. Compatible Cell Number Ranges for Preparing 50 µL and 100 µL Reactions

CELL TYPE	CELL NUMBER RANGE FOR 50 µL REACTIONS	CELL NUMBER RANGE FOR 100 µL REACTIONS
Isolated T cells	5 x 10^5 - 1 x 10^7	> 1 x 10^7 - 2.5 x 10^7
Isolated NK cells	5 x 10^5 - 1 x 10^7	> 1 x 10^7 - 2 x 10^7
CD34+ HSPCs	5 x 10^4 - 2.5 x 10^5	> 2.5 x 10^5 - 5 x 10^5
PBMCs	1 x 10^6 - 2.5 x 10^6	> 2.5 x 10^6 - 5 x 10^6

Data

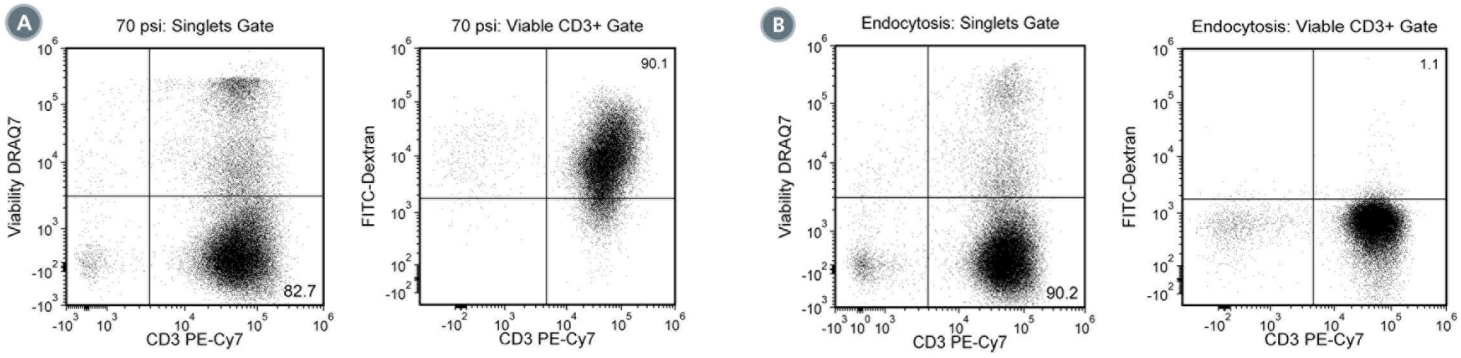

Figure 2. Starting from T cells isolated using EasySep™ Human T Cell Isolation Kit, the delivery efficiency of CellPore™ FITC-Dextran **(A)** at 70 psi is typically 92.0 ± 3.3% with an associated cell viability decrease of 8.2 ± 6.6% (mean ± SD). **(B)** Under unmanipulated conditions, the natural uptake of FITC-dextran by T cells (i.e. endocytosis) is typically below 2% (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%.

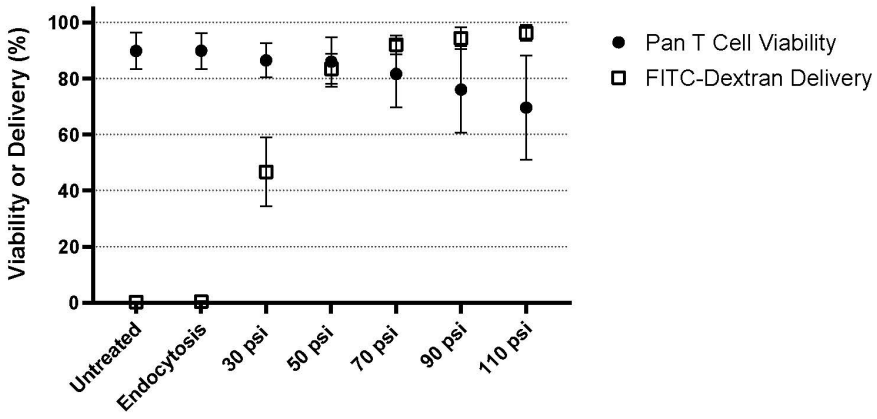

Figure 3. Representative pressure sweep graph for identification of the optimal delivery parameter (i.e. instrument pressure). CellPore™ FITC-Dextran was delivered to freshly isolated human T cells under increasing instrument pressure. Both cell viability and FITC-dextran delivery efficiency (i.e. FITC+CD3+ 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.