PBMC Boost Protocol

For research scale[1] mechanoporation of human primary PBMCs

Publication Number: PBMC I Version: A1

portal.bio

info@portal.bio

Product Description

Deliver your desired cargo[2] to a mixed population of PBMCs using Portal's Boost mechanoporation method. Mechanoporation is simple, fast, and has minimal impact on cell state, enabling immediate cell manipulation, assay, or readout.

Key factors to ensure success with mechanoporation-based cargo delivery are:

- Cell health: ensure high quality cell preparation and handling prior to mechanoporation to ensure cell health throughout the process
- Handling & timing: handle cells gently throughout the mechanoporation process to reduce cell stress; ensure an efficient workflow to reduce handling time
- Speed of transit & pore size: key factors to ensure high rates of cargo delivery are the level of cellular deformation (pore size) and speed of transit through the pore (pressure); cargo delivery is inversely related to viability as factors which increase delivery can increase shear stress

[1]50-300 µl volume

[2]Cargo options may include but are not limited to fluorescent polymers (dextran), mRNA, siRNA, RNP, proteins, peptides, and small molecules.

Materials

Supplied materials

Kit	Standard	Plus	Ultra
Gateway™ system	1	1	2
MicroBooster™ cartridge 050350520-01	10	20	30

Required materials not supplied

Material	Recommended product
Delivery buffer	Opti-Mem[3] (Gibco 31985062)
Delivery tracer	Fluorescent dextran[4]
Cargo of interest	Protein, peptide, RNA, antibody, small molecule, RNP
Tubes	50 mL, 15 mL, 1.5 mL
Plates	96-well v-bottom
Media	
R10	RPMI 1640 (Gibco 11875119) 10% FBS
Nuclease	Benzonase (Millipore Sigma E1014)

[3] Portal recommends Opti-Mem as a buffer compatible with all types of cargo. The boost process is compatible with other minimal media, serum-free complete media, or PBS. Buffer choice may impact delivery and viability. When using an RNA-based cargo, ensure the media formulation is serum and RNase free. It is not recommended to use complete media containing serum.

[4] Portal recommends co-delivering dextran in all samples as a delivery tracer. Portal's dextran of choice is 3 kDa Cascade Blue (Thermo Fisher D7132).

Cell Boosting Specifications

Parameter	Minimum	Maximum	Recommended
Volume	50 µl	300 µl	
Pressure	5 psi; 0.03 MPa	15 psi; 0.1 MPa	7-10 psi; 0.05-0.07 MPa
Cell concentration	1 x 10^7 cells/mL	1 x 10^8 cells/mL	2 x 10^7 cells/mL
Cargo concentration			
Dextran			0.1 mg/ml
mRNA			0.1 mg/ml
siRNA			10 µM
RNP			0.2 mg/ml
Cargo volume			≤ 10% total volume[5]

[5]It is not recommended to dilute the delivery buffer with the cargo buffer at a rate higher than 10% of the reaction volume, as this may alter Boost performance. If the cargo volume must exceed 10%, then PBS or NaCl may be used to balance the osmotic concentration (see protocol for details).

Protocol

Cell Preparation

1. Collect cells from culture or other preparation. This protocol is compatible with fresh or frozen PBMCs.
    - *For fresh cells:* Isolate PBMCs from a leukopak. Portal recommends using a Ficoll-based method.
    - *For cryopreserved cells:* Portal recommends freezing leukopak-isolated cells in freeze media containing 10% DMSO at a concentration of 1×10^9 cells/mL using a controlled cooling method.
        a. Flash thaw a previously frozen cryogenic tube in a 37°C water bath.
        b. Add the cells dropwise to 35 mL complete media[6] with 100 units/mL benzonase in a 50 mL conical tube. Up to 3×10^9 cells may be added per tube.
        c. Invert the tube several times to mix and incubate for 10 minutes with the tube at an angle and the cap slightly loosened in a 37°C incubator with 5% CO_2.
        d. Centrifuge cells at 200 RCF for 10 minutes. Resuspend in complete media at an appropriate culturing concentration (1×10^6 - 4×10^6 cells/mL recommended).
        e. Rest cells in an appropriate culturing vessel for 30-60 minutes in a 37 °C incubator with 5% CO_2.
2. Centrifuge cells at 400 RCF for 4 minutes to collect.
3. Resuspend cells gently in the delivery buffer at desired concentration within the range of 1×10^7 - 1×10^8 cells/mL.

[6] Portal recommends using R10, composed of RPMI 1640 + 10% FBS.

Cargo Preparation

Recommended cargo concentration

Cargo type	Recommended final concentration (1X)
Dextran	0.1 mg/mL
mRNA	0.1 mg/mL
siRNA	10 µM
CRISPR RNP	Nuclease: 0.2 mg/mL, complex with guide at a 2.5:1 guide:nuclease molar ratio

1. Ensure cargo is at room temperature.
2. Prepare cargo in a separate solution to be added to the cell suspension. There are several options for cargo preparation and addition to cells:
    a. Add cargo from a concentrated solution directly to 1X cells to achieve a final 1X concentration of cargo. Thoroughly mix by pipetting.
        i. This method is most advantageous for single cargoes in which the volume comprises ≤10% of the total reaction volume.
    b. Prepare cargo at a 2X-5X concentration in the delivery buffer to be added to a reciprocal cell solution to make 1X final for both cells and cargo when mixed. Wait to add the cargo to cells until immediately prior to boosting.
        i. Preparing a 2X cell suspension and a 2X cargo suspension is Portal's recommended procedure for complex, mixed, or sensitive cargo.
        ii. This is the recommended method if the cargo will compromise a large volume relative to the overall boost volume (≥10%).
        iii. For samples where the cargo comprises ≥10% the total reaction volume, the osmotic concentration should be rebalanced by using a 10X PBS or NaCl solution. Add the amount required to make the cargo volume at 1X salt solution (i.e. if the cargo added is 20 ul, add 2 ul 10X PBS or NaCl).

Cell Boost

See Quick Start guide for Boost Protocol.

1. Mix cells and cargo immediately prior[7] to loading the MicroBooster cartridge for optimal performance.
2. Load the cell & cargo mixture into the top of the MicroBooster cartridge.
3. Load a collection tube underneath the MicroBooster cartridge.
4. Attach the MicroBooster cartridge to the Gateway system.
5. Set the desired pressure[8] on the regulator and wait until the indicator light is ready.
6. Push the boost button and hold until the boost is complete and no more cell & cargo solution exits the MicroBooster cartridge.
    a. If the indicator light turns off prior to boost completion, the tank has de-pressurized. Release the button and allow the tank to re-pressurize and the light to turn back on before the boost can be continued, if necessary.

[7] Delaying mixing until immediately prior to Cell Boost is most important for sensitive cargo.

[8] See boosting specifications table for recommended pressure for optimal performance in PBMCs. A pressure sweep may be performed to optimize performance for specific use cases.

Cell Collection & Recovery

1. Remove the collection tube from the Gateway system.
2. At least 30 seconds following the Cell Boost, add complete cell media to the collection tube to quench the cells.
3. Spin the cells to collect and move to downstream analysis[9,10].

[9] To achieve optimal cell retention, Portal recommends spinning in a 96-well V-bottom plate and flicking to discard supernatant.

[10] If fluorescent dextran is used as a delivery tracer, cells should be washed at least once prior to downstream analysis and/or culturing to minimize background signal and cellular uptake through endocytosis in culture.

Notes and Tips

- Call us! We're happy to help (info@portal.bio)
- Results may be donor-dependent
- For optimal cell performance, experiments should be concise and completed in a short timeframe (ideally < 30 min) to reduce cell stress
- Portal recommends preparing cells & cargo as separate solutions at a 2X concentration each and mixed 1:1 prior to boosting for optimal performance
- It is ideal to add sensitive cargo (e.g., mRNA) to cells immediately prior to boosting for optimal performance
- Temperature is a variable that can affect performance. Lower temperatures are more harsh on cells but can increase delivery