Portal's Boost Specifications Sheet

PORTAL

portal.bio

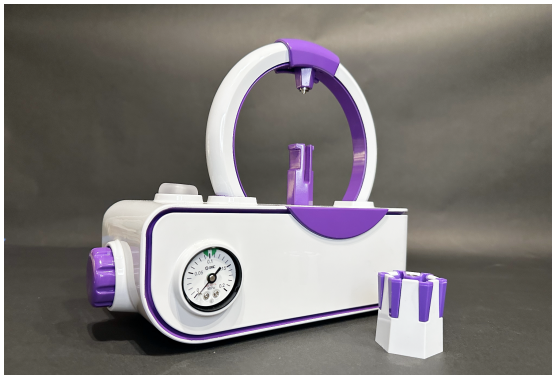
info@portal.bio

For generalized research scale[1] guidelines across a wide range of applications

Publication Number: BoostSpecs | Version: A2

Boosting Equipment

Research Scale

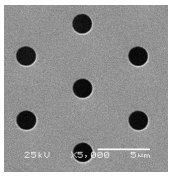


Gateway™

Pressure regulator; fits in BSC, requires outlet for power.

MicroBooster™

Boost cartridge with interior core containing cell-type-specific pores



Optimized Parameters and Recommendations

Cell Boosting Specifications

Parameter	Minimum	Maximum	Recommended
MicroBooster™ cartridge Research scale Volume (for use with Gateway™ system)	50 µl	300 µl	
Pressure	2 psi; 0.01 MPa	15 psi; 0.1 MPa	
Cell concentration	1×10^7 cells/mL	5×10^7 cells/mL	
Cargo volume			≤ 10% total volume[1]

[1]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).

Recommended Boosting Conditions

Cell type	MicroBooster™	Recommended cell concentration	Recommended Pressure
PBMCs	050350520-01	2×10^7 cells/ml (1×10^7 - 2×10^8)	7-10 psi; 0.05-0.07 MPa
Unstimulated T cells	050350520-01	2×10^7 cells/ml (1×10^7 - 5×10^7)	7-12 psi; 0.05-0.08 MPa
Activated T cells	050350624-01 050350728-01 050350832-01 050350936-01	2×10^7 cells/ml (1×10^7 - 5×10^7)	7-12 psi; 0.05-0.08 MPa
iPSCs	050351030-01 050351236-01	2×10^7 cells/ml (1×10^7 - 5×10^7)	3-7 psi; .02-.05 MPa

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

General Protocol Guidelines

Cell Preparation

1. Prepare cells cleanly and resuspend at desired concentration in the delivery buffer.
    a. Recommended range for cell concentration is 1×10^7 cells/mL - 5×10^7 cells/mL.
    b. Negative selection kits are preferred when isolating a pure population from PBMCs.
2. Cell-specific preparations:
    a. For PBMCs and isolated sub-types: following the isolation/preparation protocol, rest in culture media for 30 minutes to 1 hr prior to boosting to improve cell health.
    b. For adherent cell-types (e.g., iPSCs, fibroblasts): Dissociate cells into single cell suspension and boost soon after dissociation.
3. Cells may be prepared at a 2X concentration to be mixed with 2X cargo. This ensures balanced and accurate concentrations of all materials.

Cargo Preparation

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 µl, add 2 µl 10X PBS or NaCl).

Cell Boost

See Quick Start guide for Boost Protocol.

1. Mix cells and cargo immediately prior[2] 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 loaded MicroBooster cartridge to the Gateway system.
5. Set the desired pressure[3] 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 button 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.

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

[3]See Recommended Boosting Conditions table for a list of recommended pressures for optimal performance in each cell type. 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[4,5].

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

[5]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 cell line or 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 can affect performance. Lower temperatures are more harsh on cells but can increase delivery