Portal's Boost Guidelines & Troubleshooting

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Portal's Pro Tips

Don't see a solution to your challenges here? Contact us! We're happy to help (info@portal.bio)

Tracer Recommendation: Portal recommends using Cascade blue 3 kDa dextran (Thermo Fisher, D7132) co-delivery in all samples as a tracer for delivery efficiency

- Dextran is inert and won't react with other cargo
- Allows an immediate readout as a control for delivery performance when analyzing data with flow cytometry
- This fluorescent color fits nicely into large flow panels

Key Performance Variables: For optimal cell performance, experiments should be concise and completed in a short timeframe (ideally < 30 min) to reduce cell stress

- Good starting cell health and gentle handling protocols maintain higher performance characteristics
- Lower temperatures increase harshness & delivery
- It is ideal to add sensitive cargo (e.g., mRNA) to cells immediately prior to boosting for optimal performance. This reduces potential for sensitive cargo degradation and endocytosis of cargo by some cell types

Understanding the Principles that Drive Efficiency:

- Mechanoporation efficiency is driven by cell speed which is controlled through pressure or flow rate
- Optimization of the appropriate speed for desired efficiency may be required
- Efficiency is non-linear: as cell speed increases, efficiency increases to a plateau which allows for a range of effective conditions (speeds) which can achieve desired efficiency before excessive harshness reduces efficiency
- The delivery and viability relationship is inversely related but non-linear: as delivery efficiency increases with increasing speed, viability may drop until the plateau zone is achieved at which viability and delivery remain constant until speed becomes too harsh

Buffer Considerations:

- Delivery buffer can affect performance
    - Membrane closure is a calcium-dependent process; calcium-containing buffers will facilitate membrane closure (and cell health), while calcium-free buffers will keep membranes open for longer periods of time
    - Growth factor support can help support cell health
    - Serum is not recommended as an additive for delivery buffer
- Cargo buffer & volume can affect performance
    - High delivery is a function of proper buffer osmolarity
    - It is preferred to maintain a balanced osmolarity by ensuring cargo resuspended in low salt buffers remains <10% of the total reaction volume
    - If cargo comprises >10% of the total reaction volume, add PBS or NaCl to compensate: a 10X PBS stock solution can be used at 10% the cargo volume to balance the salt content

Recommended Buffers:

- Portal typically uses Opti-MEM (Gibco 31985062) or other minimal media for the delivery buffer
    - Opti-MEM is typically used for PBMCs and subtypes
    - Basal media is typically used for iPSCs
- Complete media supplemented with cytokines is recommended for T cell or PBMC culturing
    - Including supplements and cytokines in the media help support cell health
- mTESR Plus (StemCell 100-0276) is recommended for iPSC culturing; Supplementing with Y27632 (Rho kinase inhibitor) helps support survival following single cell workflow.

Troubleshooting Guide

Observation/ Challenge	Possible Reason	Recommended Solution
Low cell viability	Poor starting cell health	Ensure cells are handled well in processing prior to boost; follow gentle isolation and processing procedures; freeze in DMSO and thaw quickly Rest cells in complete media for 30-60 minutes prior to boosting
	Poor boost processing	Minimize time that cells are out of the incubator; ensure experiment time is ≤30 minutes for optimal performance
	Poor post-boost cell processing	Spin cells at ≤400 RCF; use flick method from plate Minimize washing & handling Recover in complete media; optimize growth factors to best support cell health & growth
	Improper conditions	Include no contact negative control (cells alone with no cargo or boost, handled in the same manner as experimental samples)
	Boost is too harsh	Decrease working pressure or increase core size Adjust cell concentration (typical optimal concentrations are 1-5 x 10^7 cells/ml
	Delivery buffer optimization	Ensure serum-free media is used; complex buffers & additives can be ok in certain cell types- try first with minimal media Minimal medias are ideal since they are simple, but can negatively impact viability; more complex media can support viability

Observation/ Challenge	Possible Reason	Recommended Solution
Low delivery	Cargo concentration is too low	Ideal cargo concentration is between 0.01 mg/ml - 1 mg/ml, with 0.1 mg/ml as a standard starting point
	Improper conditions	Include an endocytosis control sample (cells + cargo, no boost) as negative control
	Boost is too gentle	Increase working pressure or decrease core size
		Adjust cell concentration (typical optimal concentrations are 1-5 x 107 cells/ml)
	Delivery buffer optimization	Calcium-containing medias facilitate membrane closure
		Minimal medias are ideal since they are simple, but can negatively impact viability (see above)
	Improper buffer osmolarity	Use a balanced salt-containing buffer for delivery buffer
		Ensure low salt buffers are <10% of the total reaction volume, or are properly balanced by compensating with PBS or NaCl (see above)
		High salt buffers are typically not ideal for cell performance
High background signal	Too much delivery material still present	Add more wash steps prior to analysis
	Stickiness or endocytosis of cargo	Add endocytosis control sample (cells + cargo, no boost) as negative control

Observation/ Challenge	Possible Reason	Recommended Solution
Low cell retention	Cell concentration is too low	Adjust cell concentration (typical optimal concentrations are 1-5 x 10^7 cells/ml)
	Volume is retained in the Booster™ cartridge	Ensure the boost button is held down until all of the reaction volume is recovered; if the Gateway™ system de-pressurizes before all the volume has been recovered, the button may need to be pressed again

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