SARTORIUS

Product Guide

Incucyte® Neuroburst Orange Lentivirus

For Detection of Neuronal Activity in Live Cells

Product Information

Presentation, Storage and Stability

Incucyte® Neuroburst Orange Lentivirus is supplied as a 2 mL vial of 3rd generation HIV-based, VSV-G pseudotyped lentiviral particle suspended in DMEM. Material supplied is sufficient for 1 x 96-well plate.

The Incucyte® Neuroburst Orange Lentivirus should be stored at -80° C. Avoid repeated freeze-thaw cycles. Lentivirus is stable for at least 6 months from date of receipt when stored at -80° C.

Product Name	Cat. No.	Ex. Max	Em. Max	Amount	Storage	Stability
Compatible with Incucyte® Live-Cell Analysis Systems configured with a Green | Orange | NIR or an Orange | NIR Optical Module						
Incucyte® Neuroburst Orange Lentivirus	4736	558 nm	605 nm	2 mL	-80° C	6 months from date of receipt

Safety data sheet (SDS) information can be found on our website at **www.sartorius.com**

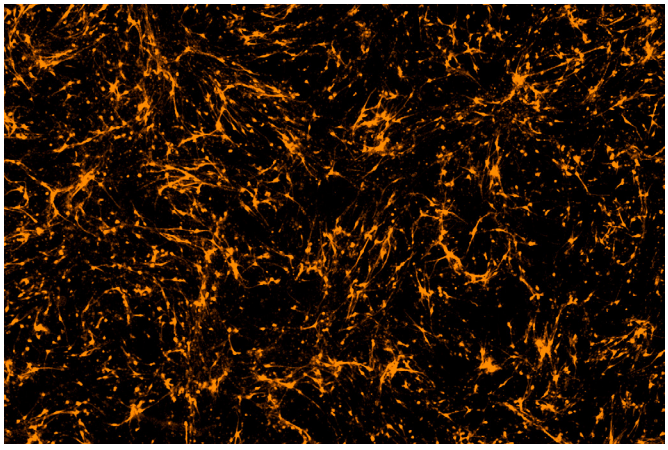
Background

The Incucyte® Neuroburst Orange Lentivirus is a lentiviral based live-cell neuronal labeling reagent driven by a synapsin promoter, resulting in the long term expression of a genetically encoded orange fluorescent calcium indicator (mRuby-based) in neuronal cells. Incucyte® Neuroburst Orange Lentivirus ensures highly efficient, yet non-disruptive labeling of primary or iPSC-derived neurons over days and weeks, and enables the kinetic quantification of neuronal activity and functional connectivity in the presence or absence of astrocytes.

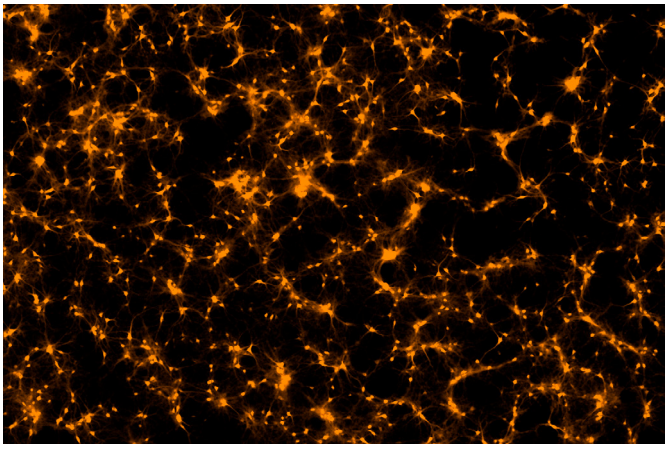
Recommended Use

We recommend thawing the Incucyte® Neuroburst Orange Lentivirus on ice and storing working aliquots at -80° C, as excessive freeze | thaw cycles can impair transduction efficiency. The lentivirus can be prepared in full media and added directly to plated cells. The Incucyte® Neuroburst Orange Lentivirus has been validated for use with the Incucyte® Live-Cell Analysis System configured with an Orange | NIR or a Green | Orange | NIR Optical Module for measurements of neuronal activity and function connectivity. The Incucyte® Neuroburst Orange Lentivirus is intended for use with the Incucyte® Neuronal Activity Analysis Software Module (Cat. No. 9600-0032).

Example Data



Fluorescent image showing iPSC derived neurons infected wth Neuroburst Orange Lentivirus (DIV 15)



Fluorescent image showing rat cortical neurons infected wth Neuroburst Orange Lentivirus (DIV 15)

Quick Guide

1. DIV: -1

Coat plate with matrix of choice and incubate at ambient temperature overnight.

2. DIV: 0

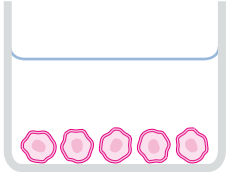


Plate rCortical Neurons.

3. DIV: 0 + 2 hours

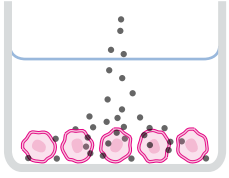
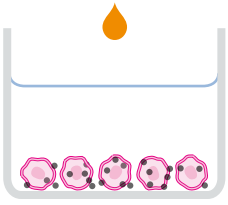


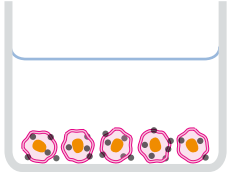
Plate rAstrocytes.

4. DIV: 2



Add Neuroburst Orange Lentivirus.

5. DIV: 3



1. Remove Neuroburst Orange Lentivirus
2. Add Uridine + 5-Fluoro-2′-deoxyuridine.
3. Start Neuronal Activity scanning in Incucyte® Live-Cell

Critical: Use rigorous aseptic technique at all times.
Only open the culture plate and medium bottles within a tissue culture hood.

Protocols and Procedures

Required Materials

- Incucyte® Neuronal Activity Analysis Software Module (Sartorius Cat. No. 9600-0032)
- Incucyte® Neuroburst Orange Lentivirus (Sartorius Cat. No. 4736)
- Incucyte® rAstrocytes (Sartorius Cat. No. 4586)
- Flat bottom 96-well tissue culture plate (e.g., Corning Cat. No. 3595)
- Water, cell culture grade
- Uridine (Sigma Cat. No. U3003)
- 5-Fluoro-2′-deoxyuridine (Sigma Cat. No. F0503)
- BrainPhys™ Neuronal Medium (Stem Cell Technologies Cat. No. 05790)
- DMEM (Invitrogen Cat. No.11965)
- Fetal Bovine Serum (Sigma Aldrich Cat. No. F2442)
- For rat cortical neurons:
  - Incucyte® rCortical Neurons (Sartorius Cat. No. 4753)
  - Poly-D-Lysine (PDL) (Millipore Cat. No. A-003-E)
  - NeuroBasal® Medium (Thermo Fisher Cat. No. 21103-049)
  - NeuroCult™ SM1 Neuronal Supplement (Stem Cell Technologies Cat. No. 05711)
  - GlutaMAX Supplement (Thermo Fisher Cat. No. 35050061)
- For iCell® GlutaNeurons:
  - Sodium borate solution (Thermo Fisher Cat. No. 28341)
  - Polyethylenimine (PEI) (Sigma Cat. No. 408727)
  - iCell® GlutaNeurons (Cellular Dynamics Cat. No. R1061)
  - Laminin (Sigma Cat. No. L2020)
  - iCell Neural Supplement B (Cellular Dynamics Cat. No. M1029)
  - N-2 Supplement (Thermo Fisher Cat. No. 1750248)
  - iCell Nervous System Supplement (Cellular Dynamics Cat. No. M1031)

Recommended Materials for Incucyte Neurite Analysis

- Incucyte® Neurotrack Analysis Software Module (Sartorius Cat. No. 9600-0010)
- Incucyte® Neurolight Orange Lentivirus (Sartorius Cat. No. 4758)

General Guidelines

- Due to differences in neuronal sources, media and culture supplements may vary. We recommend using media | supplements supplied by the specific cell vendor.
- The protocol below outlines a coating of PEI | laminin for iCell® GlutaNeurons as an example of iPSC-derived neurons and PDL for primary rat cortical neurons. Depending on neuronal source, other preferred ECM plate coatings may be used.
- Thaw no more than 1 vial of neurons at one time. Maintain neurons in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal viability and performance.
- We recommend initiating activity experiments on a Monday in order to avoid weekend feedings.

Primary Rat Cortical and Astrocyte Cell Culture Media Recipes

1. Complete Plating Medium (primary rat cortical neurons)
   - 48.5 mL Neurobasal® Medium.
   - 1 mL NeuroCult™ SM1 Neuronal Supplement.
   - Thaw one bottle of NeuroCult™ SM1 at room temperature (15–25° C) for 1 hour.

     Note: If NeuroCult™ is not used immediately, aliquot and store at -20° C. Do not exceed the expiration date (EXP) as indicated on the label.
   - 0.5 mL 100X GlutaMAX Supplement — stored at room temperature
2. Complete Maturation Medium (primary rat cortical neurons)
   - 49 mL BrainPhys™ Neuronal Medium
   - 1 mL NeuroCult™ SM1 Neuronal Supplement
   - Thaw one bottle of NeuroCult™ SM1 at room temperature (15–25° C) for 1 hour.

     Note: If NeuroCult™ is not used immediately, aliquot and store at -20° C. Do not exceed the expiration date (EXP) as indicated on the label.

     Note: Complete Plating | Maturation Media can be stored at 2–8° C for up to 2 weeks. After 2 weeks, additional fresh complete maturation medium should be made to ensure neuronal cell health and function.

     Note: See recipe for culture of iCell® GlutaNeurons on page 7.
3. Rat Astrocyte Medium
   - 425 mL DMEM
   - 75 mL Characterized FBS

     Note: If not used immediately, medium can be stored at 4° C for 3 months.

1. Plate Preparation DIV (day *in vitro*): -1

Primary Rat Cortical Neurons

1. Coat 1X 96-well plate with 100 µL/well of 0.1 mg/mL PDL in cell culture grade water.
2. Replace lid and incubate for 16–20 h at room temperature in tissue culture hood.

iCell® GlutaNeurons

We recommend plating iPSC-derived neuronal cultures on a dual coating of polyethylenimine (PEI) and laminin. *If other coatings are wanted for specific iPSC-derived neurons, customers can use what works for them.

1. Dilute 1 M Borate stock buffer solution 10-fold in dH_2O to achieve a concentration of 100 mM sodium borate solution.
2. Weigh out 0.11 g polyethylenimine and add 50 mL of 100 mM sodium borate solution.
3. Set on shaker or vortex until the PEI is completely dissolved.
4. Filter the solution through a 0.2 µM syringe filter (solution can be stored at 4° C for 7–10 days).
5. Coat 1X 96-well plate with 100 µL of PEI solution, replace lid, and incubate for 16–20 h at room temperature in tissue culture hood.

2. Plate Preparation DIV: 0

Primary Rat Cortical Neurons

1. Wash plate 3X with 200 µL cell culture grade water and let dry in tissue culture hood for approximately 1 hr (or until completely dry).

iCell GlutaNeurons

1. Using a vacuum manifold or multi-channel pipette, completely remove PEI from all wells.
2. Wash plate 3X with 200 µL sterile cell culture grade water and let dry in tissue culture hood for approximately 1 h, ensuring no residual liquid remains in wells.
3. Laminin coating:
   a. Thaw 1 mg/mL laminin at 4° C to prevent premature gelling.
   b. When thawed, dilute 1 mg/mL laminin solution 1:300 in sterile culture grade water to a final concentration of 3.3. µg/mL immediately before use. Do not vortex the solution.
   c. Add 100 µL into each well and incubate at least 1 h at 37° C.
   d Remove laminin just before seeding cells. Seed each column separately to avoid drying of laminin coating.

3. Seeding of Neurons DIV: 0

Primary Rat Cortical Neurons

We recommend initial seeding in Complete Plating Medium and continued culture in Complete Maturation Medium (see included recipes).

Note: Equilibrate Complete Plating Medium to ambient temperature before thawing cells.

1. Thaw no more than 1 vial of neurons by immersing the cryovial in a 37° C water bath for approximately 2–3 min (avoid submerging the cap), holding the tube stationary (no swirling).
2. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in tissue culture hood.
3. Pre-wet a sterile 50 mL centrifuge tube by addition and removal of 1 mL Complete Plating Medium.
4. Pre-wet P1000 tip with Complete Plating Medium and gently transfer the cryovial contents to the pre-wet 50 mL centrifuge tube.
   Note: Use of a 50 mL centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase neuron viability.
5. Rinse the empty cryovial with 1 mL of Complete Plating Medium to recover any residual cells from the vial.
6. Transfer the 1 mL of Complete Plating Medium from the cryovial drop-wise (~ 1 drop/sec) to the 50 mL centrifuge tube containing the neuronal suspension. Gently swirl the tube while adding the Complete Plating Medium to mix the solution completely and minimize osmotic shock on the thawed cells.
7. Slowly add an additional 3 mL of Complete Plating Medium to the 50 mL centrifuge tube drop-wise (~ 1–2 drops/sec). Gently swirl the centrifuge tube while adding the Complete Plating Medium.
   Note: It is critical to add the Complete Plating Medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.
8. Count live neurons with Trypan Blue (10 µL cell suspension + 10 µL Trypan Blue) using a hemocytometer. Adjust cell stock to 133,333 cells/mL to seed 20,000 neurons in 150 µL per well, using Complete Plating Medium.
9. Add 150 µL mixed cell suspension into each well (20,000 cells/well)
   Note: While seeding neurons, gently mix cell suspension with a multichannel pipettor.
10. Let plate sit at room temperature in tissue culture hood for approximately 20 min to allow neurons to settle evenly in the wells.
11. Incubate plate(s) for approximately 2 h at 37° C before addition of astrocytes.

iCell GlutaNeurons

We recommend plating and keeping iCell GlutaNeurons in Complete BrainPhys Medium (see included recipe).

Note: Equilibrate Complete BrainPhys Medium to ambient temperature before thawing cells. We recommend a cell density of 20,000 cells per well, but this may need to be optimized for other iPSC-derived neuronal cell types.

1. Thaw no more than 1 vial of neurons by immersing the cryovial in a 37° C water bath for approximately 2–3 min (avoid submerging the cap), holding the tube stationary (no swirling).
2. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in tissue culture hood.
3. Pre-wet a sterile 50 mL centrifuge tube by addition and removal of 1 mL Complete BrainPhys Medium.

4. Pre-wet P1000 tip with Complete BrainPhys Medium and gently transfer the cryovial contents to the pre-wet 50 mL centrifuge tube.
   Note: Use of a 50 mL centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase neuron viability.
5. Rinse the empty cryovial with 1 mL of Complete BrainPhys Medium to recover any residual cells from the vial.
6. Transfer the 1 mL of Complete BrainPhys Medium from the cryovial drop-wise (~ 1 drop/sec) to the 50 mL centrifuge tube containing the neuronal suspension. Gently swirl the tube while adding the Complete BrainPhys Medium to mix the solution completely and minimize osmotic shock on the thawed cells.
7. Slowly add an additional 8 mL of Complete BrainPhys Medium to the 50 mL centrifuge tube drop-wise (~ 1–2 drops/sec). Gently swirl the centrifuge tube while adding the Complete BrainPhys Medium.
8. Centrifuge the cell suspension at 400 x g at room temperature for 5 min.
9. Resuspend the cell pellet in 3 mL Complete BrainPhys Medium by gentle trituration (~ 30 sec).
   Note: It is critical to add the Complete BrainPhys Medium slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.
   Note: Resuspension volume may need to be adjusted for other iPSC-derived neuronal cell types, depending on cell number.
10. Count live neurons with Trypan Blue (10 µL cell suspension + 10 µL Trypan Blue) using hemocytometer. Adjust cell stock to 133,333 cells/mL to seed 20,000 neurons in 150 µL per well, using Complete BrainPhys Medium.
11. Add 150 µL mixed cell suspension into each well (20,000 cells/well)
    Note: While seeding neurons, gently mix cell suspension with a multichannel pipettor.
12. Let plate sit at room temperature in tissue culture hood for approximately 20 min to allow neurons to settle evenly in the wells.
13. Incubate plate(s) for approximately 2 h at 37° C before addition of astrocytes.

4. Seeding of Primary Rat Astrocytes for both Primary Rat Cortical Neurons and iCell GlutaNeurons DIV: 0 + 2 hours

Note: We recommend using rAstrocytes for both rCortical Neurons and iPSC co-cultures to maintain cell viability. Equilibrate astrocyte medium to ambient temperature before thawing cells.

1. Thaw 1 vial of rAstrocytes by immersing the cryovial in a 37° C water bath for approximately 2–3 min (avoid submerging the cap), holding the tube stationary (no swirling).
2. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in tissue culture hood.
3. Pre-wet a sterile 50 mL centrifuge tube by addition and removal of 1 mL astrocyte medium.
4. Pre-wet P1000 tip with astrocyte medium and gently transfer the cryovial contents to the pre-wet 50 mL centrifuge tube.
   Note: Use of a 50 mL centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase astrocyte viability.
5. Rinse the empty cryovial with 1 mL of room temperature culture medium to recover any residual cells from the vial.
6. Transfer the 1 mL of astrocyte medium from the cryovial drop-wise (~ 1 drop/sec) to the 50 mL centrifuge tube containing the neuronal suspension. Gently swirl the tube while adding the astrocyte medium to mix the solution completely and minimize osmotic shock on the thawed cells.
7. Slowly add an additional 3 mL of astrocyte medium to the 50 mL centrifuge tube drop-wise (~ 1–2 drops/sec). Gently swirl the centrifuge tube while adding the astrocyte medium.
8. Centrifuge the cell suspension at 250 x g for 5 min at room temperature.
9. Carefully aspirate the supernatant leaving approximately 0.5 mL astrocyte medium in centrifuge tube. Resuspend cells in 5 mL astrocyte medium.
10. Count astrocytes with Trypan Blue (10 µL cell suspension + 10 µL Trypan Blue) using hemocytometer. Adjust cell stock to 300,000 cells/mL using astrocyte medium and gently seed 15,000 astrocytes in 50 µL per well (total volume per well should now be 200 µL) of 96-well plate containing neurons.
    Note: While seeding astrocytes, gently mix cell suspension with a multichannel pipettor.

5. Infection of neurons with Incucyte® Neuroburst Orange Lentivirus DIV: 2

We recommend infection of neuronal cultures on DIV 2. However, later infections can be done without alterations in expression levels.

Note: Incucyte® Neurolight Orange Lentivirus can also be used on a separate plate for expression control.

1. Thaw Neuroburst Orange Lentivirus on wet ice (approximately 1–2 hours).
2. Dilute Neuroburst Orange Lentivirus in Complete Plating Medium for primary rat cortical neurons, Complete BrainPhys Medium for iCell GlutaNeurons or appropriate medium for other iPSC-derived neuronal cell types needed for a final addition volume of 100 uL/well.
   Note: See optimization recommendations at end of protocol for determining the appropriate amount of virus to use for the neuronal cell type of interest.

3. Remove 100 µL Complete Plating Medium for primary rat cortical neurons, Complete BrainPhys Medium for iCell GlutaNeurons or appropriate medium for other iPSC-derived neuronal cell types from the assay plate containing the neuronal co-cultures.
4. Using a multichannel pipettor, gently add 100 µL/well of diluted Neuroburst Orange Lentivirus prepared above to each well of your cell plate. Do not mix and return to incubator immediately.

   Critical: Do not pipette up and down after adding the virus solution as this may result in damage to the plated neurons.

6. Remove Neuroburst Orange Lentivirus | 5-FDU/U Treatment DIV: 3

1. Prepare 90 mL of 5-F-DUridine/Uridine (5-FDU/U).
   a. Weigh 7.2 mg 5-FDU (90 mL x 0.08 mg/mL = 7.2 mg).
   b. Weigh 25.2 mg U (90 mL x 0.28 mg/mL = 25.2 mg).
   c. Dissolve in 90 mL Complete Plating Medium for primary rat cortical neurons, Complete BrainPhys Medium for iCell GlutaNeurons or appropriate medium for other iPSC-derived neuronal cell types, and sterile filter.
   d. Make 4 mL aliquots and store at -20° C.
2. Remove 190 µL of medium from the assay plate containing neuronal | astrocyte co-cultures and replace with 90 µL of Complete Plating Medium for primary rat cortical neurons, Complete BrainPhys Medium for iCell GlutaNeurons or appropriate medium for other iPSC-derived neuronal cell types.
3. Add 100 µL/well of 2X 5-FDU/U for a final assay concentration of 8 µg/mL and 28 µg/mL, respectively, to inhibit astrocyte proliferation.

   Note: If infection is performed at a later time point, add 5-FDU/U at DIV3 and remove Neuroburst Orange Lentivirus (as described) 24 h after infection.

7. Scan Set-Up DIV: 3

Neuronal Activity Plate

1. Place the cell plate into the Incucyte® Live-Cell Analysis System and allow to warm to 37° C for 20–30 min prior to scanning (typical scan pattern is 3 min/well, 1 scan/day).
   a. Scan type: Neuronal Activity
   b. Channel selection: Phase and Orange
   c. Movie acquisition time: 30 s–180 s (default is set to 180 s)
   d. Objective: 4X
   e. Scan interval: Every 24 h
2. Perform 50% media exchange (100 µL) with Complete Maturation Medium for primary rat cortical neurons, Complete BrainPhys Medium for iCell GlutaNeurons or appropriate medium for other iPSC-derived neuronal cell types 3X per week.

Neurotrack Plate

1. Place the cell plate into the Incucyte® Live-Cell Analysis System and allow to warm to 37° C for 20–30 min prior to scanning.
   a. Scan type: Standard
   b. Channel selection: Phase and Orange
   c. Objective: 20X
   d. 4 images per well
   e. Scan interval: Every 6 h
2. Perform 50% media exchange (100 µL) with Complete Maturation Medium for primary rat cortical neurons or Complete BrainPhys Medium for iCell GlutaNeurons 3X per week.

8. Feeding Cultures DIV: 6

Primary Rat Cortical Neurons

1. Make Complete Maturation Medium (see recipe above) and warm in a 37° C water batch.

   Note: The Complete Maturation Medium will be used during the remainder of the assay.
2. Perform a 50% (100 µL) media exchange with Complete Maturation Media 3X per week.

iCell GlutaNeurons

1. Use Complete BrainPhys Medium for iCell GlutaNeurons or optimized culture medium for other iPSC-derived neurons being used and perform 50% (100 µL) medium changes 3X per week.

Incucyte® Neuroburst Orange Lentivirus Optimization

Note: We recommend optimizing Neuroburst Orange Lentivirus volume per well for each uncharacterized cell type tested. Quality control for the Incucyte Neuroburst Orange Lentivirus is the ability to efficiently infect Incucyte rCortical Neurons to express the mRuby-based Incucyte Neuroburst Orange Lentivirus driven off of the synapsin promoter, such that a concentration of > 3.7 µL/20,000 neurons results in an active object count > 500 at day 10 in a Neuronal Activity Assay (rCortical Neurons | rAstrocyte co-culture experiment). We recommend performing a volumetric titration from 100–0.14 µL for each neuronal cell line evaluated. The lowest concentration that results in the highest count of active objects should be selected. Evaluation of neuronal activity is to be performed on an Incucyte® Live-Cell Analysis System.

1. Thaw reagent on wet ice: Neuroburst Orange Lentivirus in DMEM (reagent is stored at -80° C).
2. To find the optimal reagent volume, we recommend testing a range of 100 µL to 0.14 µL per well from at least 4 wells for each plating density of neurons tested.

   Note: We have found 20,000 neurons/well to be a good starting point for many cell types.
3. In a sterile 96-well culture plate, create serial dilutions of Neuroburst Orange Lentivirus using the provided plate map (Figure 1).
   a. Add 180 µL of Neuroburst Orange Lentivirus to wells A4-A7.
   b. Add 120 µL of appropriate medium to wells B4-B7 and continue down the entire plate to wells to wells H4-H7.
   c. Perform a 1:3 serial dilution by transferring 60 µL of Neuroburst Orange Lentivirus from wells A4-A7 to wells B4-B7 and continue down the plate, stopping at row G. Row H is a no virus control and contains only medium.
4. We recommend using the Incucyte Neurolight Orange Lentivirus for expression control on a separate plate. The same dilution protocol should be used as Neuroburst Orange Lentivirus optimization.

   Note: Neurolight Orange Lentivirus expression control optimization must be conducted on a separate plate, as a different scan type is used (Standard).
5. Once virus dilution plate is created, use a multichannel pipettor to gently add 100 µL/well of diluted Neuroburst Orange Lentivirus prepared above to each well of your cell plate.

   Note: Do not mix and return to incubator immediately.

iCell GlutaNeurons Cell Culture Medium Recipe

iCell GlutaNeurons are cultured in Complete BrainPhys Medium comprised of BrainPhys Neuronal Medium, iCell® Neural Supplement B, iCell® Nervous System Supplement and N-2 supplement. The Complete BrainPhys Medium is serum-free and has been specially formulated to maintain the health and function of iCell GlutaNeurons while limiting the proliferation of progenitor or non-neuronal cells. iCell GlutaNeurons can be maintained in culture for at least 2 weeks in this medium without appreciable loss of viability or purity. For 200 mL:

- BrainPhys™ Neuronal Medium (192 mL)
- iCell Neural Supplement B (4 mL)
- iCell Nervous System Supplement (2 mL)
- N-2 Supplement (2 mL)

1. Thaw iCell Neural Supplement B, iCell Nervous System Supplement, and N-2 supplement at room temperature on the day of medium preparation.
2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Using sterile technique, add the entire contents of the iCell Neural Supplement B vial (~ 2 mL), iCell Nervous System Supplement vial (~ 1 mL), and N-2 supplement (1 mL) to the BrainPhys Neuronal Medium (96 mL) to make the Complete BrainPhys Medium. Filter the Complete BrainPhys Medium through a 0.22 µm sterile filter unit.
4. Store the Complete BrainPhys Medium at 4° C, protected from light, for up to 2 weeks.

   Note: We recommend using room temperature Complete BrainPhys Medium to thaw iCell GlutaNeurons.

   Note: Freeze remaining N-2 supplement in 1 mL aliquots. Do not refreeze the other individual medium components or Complete BrainPhys Medium.

	1	2	3	4	5	6	7	8	9	10	11	12
A				Neuroburst Orange Lentivirus 100 µL/well								
B				Neuroburst Orange Lentivirus 33.33µL/well								
C				Neuroburst Orange Lentivirus 11.11 µL/well								
D				Neuroburst Orange Lentivirus 3.7 µL/well								
E				Neuroburst Orange Lentivirus 1.23 µL/well								
F				Neuroburst Orange Lentivirus 0.41 µL/well								
G				Neuroburst Orange Lentivirus 0.14 µL/well								
H				No Virus Control								

Figure 1

Determining optimal Neuroburst Orange Lentivirus volume

- It is important to look at all metrics related to activity and connectivity when selecting the optimal volume of Neuroburst Orange Lentivirus.
- The optimal amount will be the lowest volume producing the highest active object count while not affecting the other metrics of connectivity and activity.
- Figure 2 shows the results of a Neuroburst Orange Lentivirus optimization experiment on iPSC-derived neurons. Active object count, mean correlation, mean burst rate, and mean burst strength are shown.
- The 100 and 33 µL per well concentrations show the highest number of active objects. These concentrations do not impact correlation measurements of network connectivity, and they show the highest level of burst strength. However, the burst rate is reduced when compared to lower concentrations in the time period between day 8 and 10.
- The 11 µL/well gives a sufficient active object count without significantly altering other metrics.
- This effect on burst rate can be observed by looking at the activity summary traces for the day 9 timepoint. Examples of these traces are shown in Figure 3.

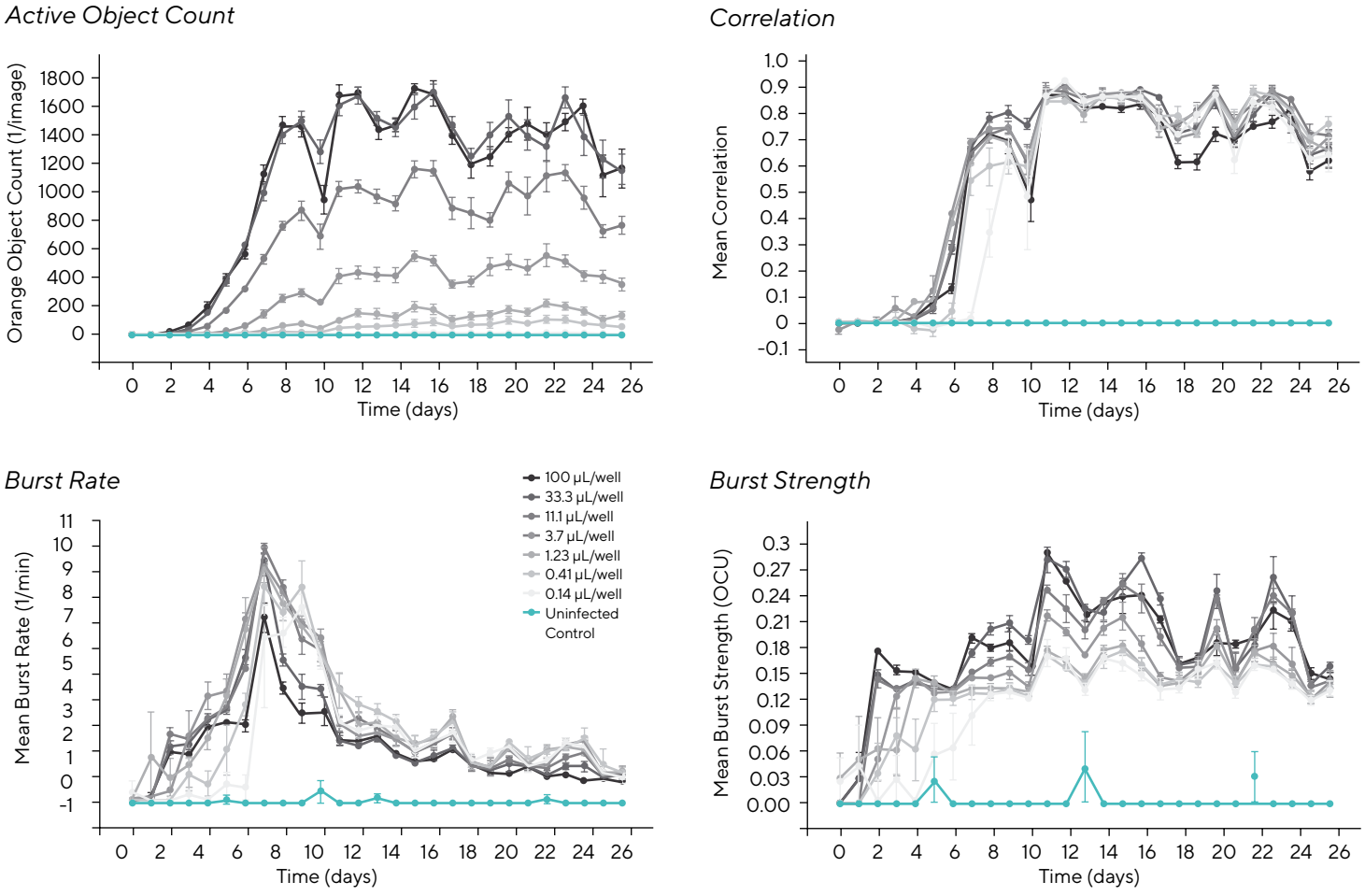


Figure 2

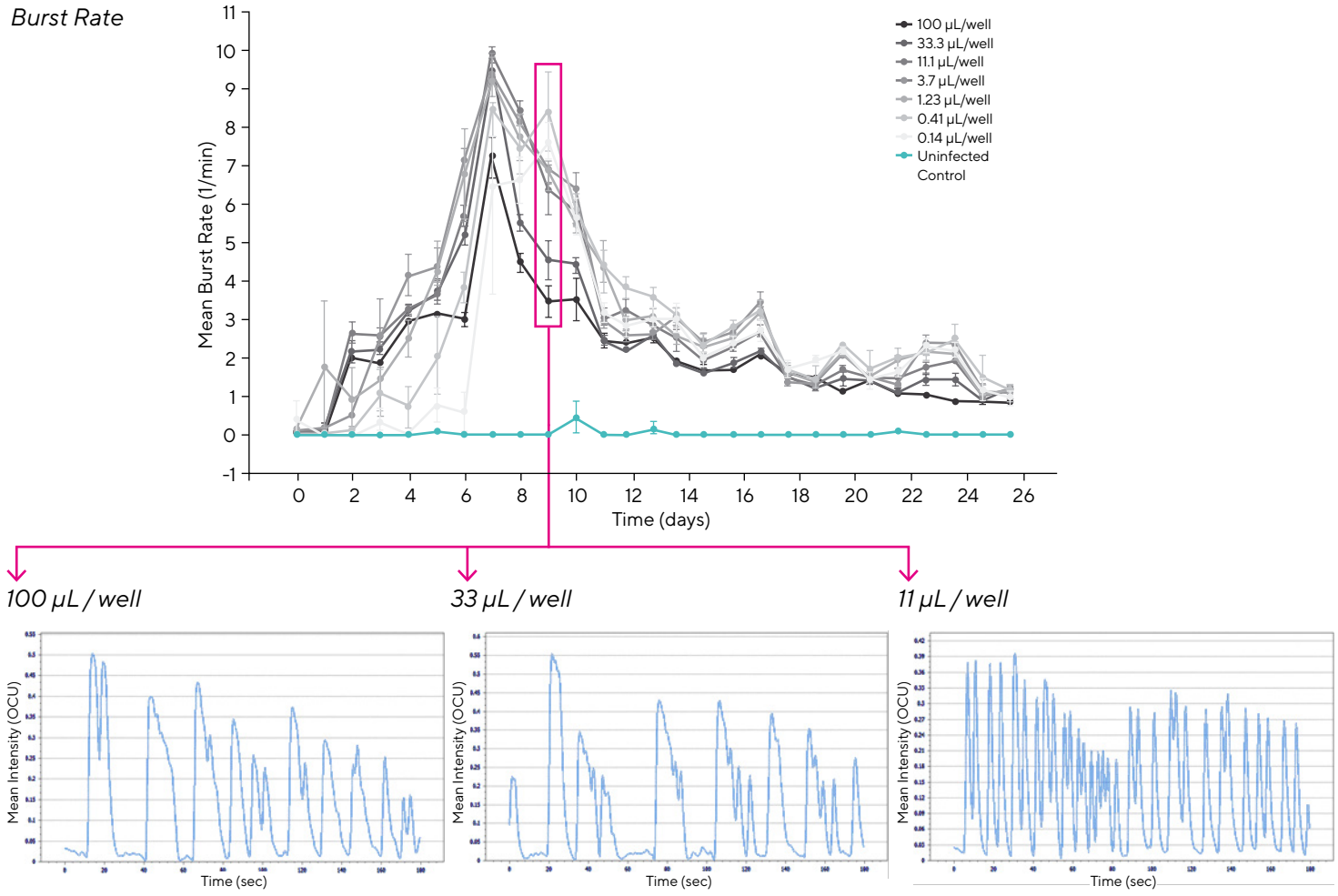


Figure 3

Safety Considerations

Sartorius products are high-quality reagents and materials intended for research purposes only; not for therapeutic or diagnostic use.

These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling lentivirus reagents. Please read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

The backbone of the lentivirus particles in this system has been modified to improve their safety and minimize their relation to the wild-type human HIV-1 virus.

These modifications include:

- The lentiviral particles are replication incompetent and only carry the non-oncogenic gene of interest.
- A deletion in the 3′ LTR (U_3) results in "self-inactivation" (SIN) of the lentivirus after transduction and genomic integration of the target cell (Yee *et al.*, 1987; Yu *et al.*, 1986; Zufferey *et al.*, 1998). This alteration renders the lentiviral genome incapable of producing packageable virus following host integration.
- The envelope is psueudotyped with the VSV-G gene from Vesicular Stomatitis Virus of the HIV-1 envelope (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).

Replication-defective lentiviral vectors, such as the 3rd generation vector provided in this product, are not known to cause any diseases in humans or animals. However, lentivirus particles still pose some biohazardous risk because they can transduce primary human cells and can integrate into the host cell genome, thus posing some risk of insertional mutagenesis. For this reason, we highly recommend that you treat lentiviral stocks as Biosafety Level 2 (BSL-2, BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.

For more information about the BL-2 guidelines and lentivirus handling, we recommend referring to local documentation based on geography. The Essen BioScience 3rd generation HIV-based lentiviruses meet BL-2 requirements based on the criteria in the document, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Institutional Guidelines: Safety requirements for use and handling of lentiviruses may vary at individual institutions. We recommend consulting your institution's health and safety guidelines and/or officers prior to implementing the use of these reagents in your experiments.

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