

Quick-Neuron™ Cholinergic - SeV Kit (Small)

Catalog Number: CH-SeV-S

Introduction

The Quick-Neuron™ Cholinergic - SeV Kit (Small) facilitates rapid and efficient differentiation of human iPS or ES cells into a population of primarily cholinergic neurons in just 6 days. Our proprietary transcription factor-based stem cell differentiation method uses Sendai virus to produce highly pure populations of neurons without a genetic footprint. Quick-Neuron™ Cholinergic differentiated cell cultures display typical neurite outgrowth and express a variety of neuronal markers, such as the pan-neuronal marker tubulin beta 3 class III (TUBB3) and the cholinergic marker choline acetyl-transferase (ChAT). When handled and maintained according to the instructions in this user guide, cholinergic neurons are viable long-term and are suitable for a variety of characterization and neurotoxicity assays.

Scale: The Quick-Neuron™ Cholinergic - SeV Kit (Small) contains a set of reagents for use with a total of 4 wells of a 24-well plate.

Related Products: Quick-Neuron™ Cholinergic - mRNA Kit, Catalog Number: CH-mRNA
Quick-Neuron™ Cholinergic - Human iPSC-derived Neurons, Catalog Number: CH-SeV-CW
Quick-Neuron™ Cholinergic - Maintenance Medium, Catalog Number: CH-MM

Kit Contents

Upon receipt, store the reagents at the temperatures indicated in the table below. All reagents are shipped on dry ice.

Reagents	Volume	Storage
QN-SeV*	55 µl	-80°C
Component N1	830 µl	-20°C or -80°C
Component A	38 µl	-20°C or -80°C
Component P	14 µl	-20°C or -80°C
Coating Material A	15.7 µl	-20°C or -80°C

This kit contains iMatrix-511 silk (Nippi, Inc.).

***IMPORTANT!** This kit contains Sendai virus (SeV) particles that are active at 33°C and become inactive at 37°C. SeV is non-pathogenic in humans, and humans are not natural hosts of SeV; however, Biosafety Level 2 (BSL-2) containment is required for its use. Please use a biological safety cabinet, laminar flow hood, and proper personal protective equipment in order to prevent mucosal exposure. More information on BSL-2 guidelines can be found at www.cdc.gov/labs/BMBL.html.

Conditions of Use

This product is for research use only. It is not approved for use in humans or for therapeutic or diagnostic use.

Technical Support

For technical support, please contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Required Consumables

Item	Vendor	Catalog Number
24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
TrypLE Select Enzyme (1X)*	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Poly-L-Ornithine**	Sigma-Aldrich	P4957-50ML
Extracellular Matrix such as** • Laminin Mouse Protein, Natural, or • Geltrex Basement Membrane Matrix	ThermoFisher	23017015 or A15696-01
Phosphate-buffered saline (without Ca ⁺⁺ Mg ⁺⁺)	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
12-mm glass coverslips***	VWR	89167-106
100% Ethanol	Multiple vendors	

* Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

** Can be substituted with our Neuron Coating Solutions (Coating Materials B and C), Catalog Number: NCS.

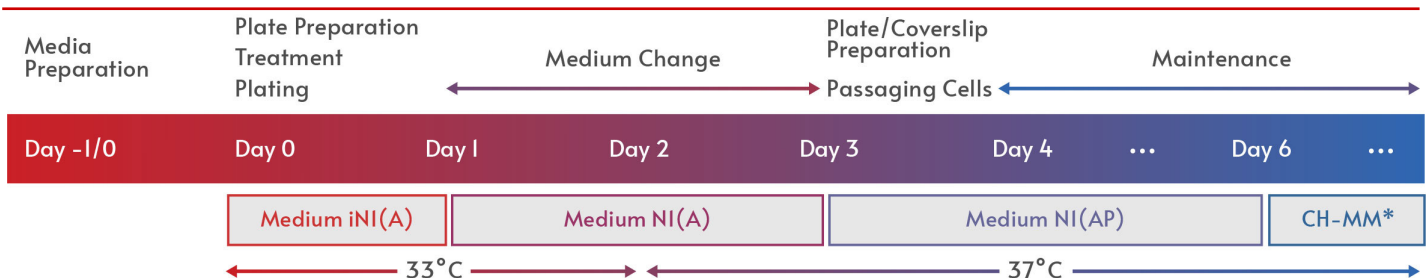
*** Glass coverslips from different brands might have different effects on the growth of neural cells. We recommend using glass coverslips from Chemglass for the best results.

Source hPSC Culture Conditions

The Quick-Neuron™ Cholinergic - SeV Kit (Small) gives the best differentiation results when source hPSCs have been maintained in StemFit® Basic04, StemFlex™ Medium, or other similar culture media which enable the maintenance of cultures by single-cell passaging. This protocol also assumes that the source hPSCs are cultured in a 35-mm culture dish or one well of a 6-well plate. If iMatrix-511 silk is routinely used as a coating substrate, prepare one culture dish or well precoated with 0.25 µg/cm² iMatrix-511 silk diluted in 2 ml chilled PBS for this kit.

- The protocols and reagents for StemFit® Basic04 and iMatrix-511 silk culture conditions are available at Elixirgen Scientific (Catalog Numbers: ASB04, NI511S).
- Differentiation should not be performed until the cells are at least 14 days post-thaw.

Workflow



*From Day 6, users may maintain differentiated neurons in the maintenance medium best suited for their needs, though we recommend Quick-Neuron™ Cholinergic - Maintenance Medium, Catalog Number: CH-MM.

Media Preparation

10 mM ROCK inhibitor Y27632 (iROCK)

1. Dissolve 10 mg ROCK inhibitor Y27632 in 3.12 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution is hereafter referred to as iROCK and can be stored at -20°C.

0.5X TrypLE Select with EDTA (Solution D1)*

1. Mix 1 ml TrypLE Select Enzyme (1X) with 1 ml 0.02% EDTA in DPBS.
2. This mixture (hereafter referred to as Solution D1) can be stored at 4°C for 2 weeks.

*Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

0.002% Poly-L-Ornithine solution (ornithine)*

1. Take 300 µl 0.01% Poly-L-Ornithine solution and mix it with 1.2 ml PBS.
2. Store the resulting 0.002% Poly-L-Ornithine solution (hereafter referred to as ornithine) for up to 2 weeks at 4°C.

*Ornithine can be substituted with Coating Material B from our Neuron Coating Solutions, Catalog Number: NCS.

1 mg/ml laminin stock solution (laminin)*

1. Thaw Laminin Mouse Protein, Natural and chill PBS at 4°C or on ice.
2. Mix the Laminin Mouse Protein, Natural and PBS to make the 1 mg/ml stock solution (hereafter referred to as laminin).
 - Laminin concentration varies by lot, so use the number specified on the vial or CoA when making your calculations.
3. Make aliquots of a convenient volume (e.g., 15 µl) and store at -20°C.

*Laminin can be substituted with Coating Material C from our Neuron Coating Solutions, Catalog Number: NCS.

Medium N1(A)

1. Prepare Medium N1(A) using the reagents listed in the table below.
 - Thaw Component N1 on ice for 20-30 minutes.
 - Thaw all other reagents at room temperature for 20-30 minutes.
2. Store Medium N1(A) for up to 2 weeks at 4°C. The leftover reagents can be discarded or saved for other uses.

Medium N1(A) Reagents	Volume
DMEM/F12	7 ml
Neurobasal Medium	7 ml
200 mM Glutamax (100x)	73 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	146 µl
Component N1	438 µl
Component A	14.6 µl

Medium N1(AP)

1. Prepare Medium N1(AP) by mixing together the following components in a 15 ml conical tube.
 - Warm Medium N1(A) at room temperature for 20-30 minutes.
 - Thaw Component P at room temperature for 20-30 minutes.
2. Store Medium N1(AP) for up to 2 weeks at 4°C

Medium N1(AP) Reagents	Volume
Medium N1(A)	6.5 ml
Component P	3.3 µl

Plate Preparation

1. Prepare diluted Coating Material A by mixing together the following components in a 15 ml conical tube.
 - Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
 - Make sure chilled PBS is used for this mixture.

Diluted Coating Material A Reagents	Volume
Coating Material A	11.9 μ l
Chilled PBS	3.6 ml

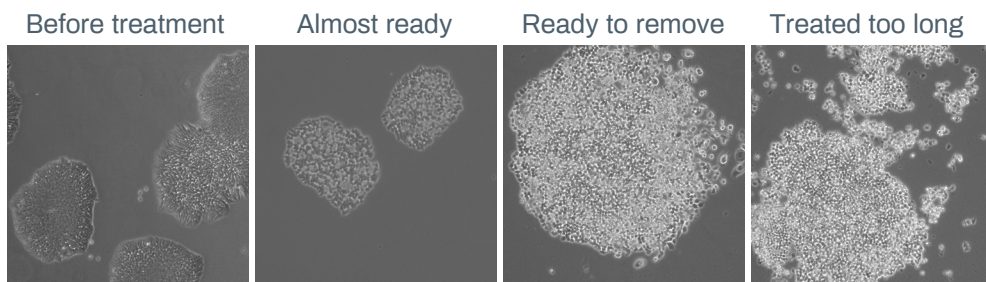
2. Add 400 μ l diluted Coating Material A to each new well of 4 wells.
3. Incubate the plate at 37°C, 5% CO₂ for 2 hours (or 4°C overnight one day before Day 0).
4. Aspirate the supernatant from each well and add 500 μ l PBS.
5. Incubate the plate at 37°C, 5% CO₂ until the hPSCs are ready for plating.

Treatment

1. Prepare Medium iN1(A) by mixing together the following components in a 15 ml conical tube.
 - Warm Medium N1(A), iROCK, and Solution D1 at room temperature for at least 1 hour.
 - The rest of Medium N1(A) should be stored at 4°C for later use.

Medium iN1(A) Reagents	Volume
Medium N1(A)	2.5 ml
iROCK	2.5 μ l

2. Thaw QN-SeV on ice.
3. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
4. Aspirate old medium from hPSC culture and add 2 ml PBS.
5. Rock the plate 3 times, aspirate PBS from the culture, and add 300 μ l of the cell dissociation reagent Solution D1. Keep the rest of Solution D1 at 4°C for use on Day 3.
6. Incubate the culture at 37°C, 5% CO₂ for 5 minutes. If all the cells are not rounded under a microscope, incubate at 37°C, 5% CO₂ for up to 5 more minutes in 1-2 minute increments (see images below).



7. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iN1(A) to the well.
8. Disperse the medium over the bottom surface of the well by pipetting 8-15 times to detach cells.
9. Using the same pipette tip, collect the cell suspension in a 1.5 ml tube.

IMPORTANT! In this protocol, users will treat hPSCs with QN-SeV in a tube and then plate the cells onto 4 wells with 250 μ l Medium iN1(A) (0.5×10^5 cells) per well. However, we recommend preparing a suspension of 1.1 ml to avoid insufficiency. First, QN-SeV should be mixed with 110 μ l of a dense cell suspension to increase the chance that QN-SeV finds its host cells. After 10 minutes incubation at 33°C, the total volume will be brought up to 1.1 ml with Medium iN1(A). Cell count may vary based on cell health, the method, and instrument used for cell counting.

10. Count cells to determine the volume of cell suspension needed for 4 wells and include a 10% buffer (a total of 2.2×10^5 cells to plate 0.5×10^5 cells in each of the 4 wells). Transfer the determined volume of the cell suspension into a 15 ml conical tube. Adjust the volume to 110 μ l with Medium iN1(A). If the volume of the cell suspension needed to get 2.2×10^5 cells exceeds 110 μ l, centrifuge the required volume of cell suspension at 200xg for 4 minutes, remove the supernatant, and resuspend the pellet into 110 μ l Medium iN1(A).

IMPORTANT! Before adding QN-SeV, ensure that it is fully thawed. Do not centrifuge, vortex, or mix SeV with a pipettor; SeV is highly sensitive to physical stress.

11. Add 55 µl QN-SeV to the hPSCs and mix them by tapping with finger 2-3 times. Cap the tube loosely to allow gas exchange.
12. Incubate the cell suspension at 33°C, 5% CO₂ for 10 minutes with intermittent mixing, by finger tapping, every 2 minutes.

Plating

1. Bring up the volume of cell suspension to 1.1 ml with Medium iN1(A).
2. Aspirate PBS from only one coated well at a time and add 250 µl cell suspension to the well. Most of the PBS should be aspirated but not completely to prevent the coated wells from drying before adding the cell suspension. Likewise, the cell suspension should be added to the well immediately after PBS is removed. Handle one well after another.
3. Move the plate in 5 cycles of quick back-and-forth and side-to-side motions to evenly distribute treated cells in the cultures.
4. Incubate the cultures at 33°C, 5% CO₂ overnight.

Day 1



Medium Change

1. Warm Medium N1(A) at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor and add 500 µl Medium N1(A).
3. Incubate the cultures at 33°C, 5% CO₂ overnight.

Day 2



Medium Change and Temperature Shift

1. Warm Medium N1(A) at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor and add 500 µl Medium N1(A).
3. Incubate the cultures at 37°C, 5% CO₂ overnight.

Day 3



Plate/Coverslip Preparation

IMPORTANT! Cells can be plated on glass coverslips or in wells of a 24-well plate depending on the desired application. Wells are better suited for functional assays, whereas coverslips work best for immunostaining and imaging. If using glass coverslips, follow all instructions starting at Step 1. If not, start at Step 1 and skip Steps 2 and 3. For first time users, we recommend only passaging two of the cultures and maintaining the other cultures (or any cultures less than 40% confluent) without passaging. Prepare one coverslip or well for each well being passaged.

1. Warm Medium N1(AP) and thaw ornithine* at room temperature for 20-30 minutes.
2. (Coverslip only) Soak 12-mm glass coverslips and the tips of forceps in 100% ethanol for 3 minutes.
3. (Coverslip only) One by one, air dry each coverslip for 1 minute or until completely dry and put it into a well of the 24-well plate using the sterilized forceps.
4. Vortex ornithine briefly and centrifuge it at a maximum speed for a few seconds.
5. Add 300 µl ornithine to the surface of each glass coverslip or well.
 - **IMPORTANT!** If using coverslips, ensure they are fully submerged under solutions from Step 5 onwards.
6. Incubate the plate at 37°C, 5% CO₂ for at least 2 hours (or at 4°C overnight one day before plating).
7. Thaw laminin* and chill 1.5 mL PBS on ice for 20-30 minutes.
8. Add 15 µl laminin to the chilled PBS. Mix well.
 - All PBS washes should be done dropwise and with room temperature PBS. Chilled PBS is only for the coating step.
9. Aspirate the supernatant from each coverslip/well and add 500 µl PBS.
10. Repeat Step 9.

11. Aspirate PBS from each coverslip/well and add 300 μ l diluted laminin.
12. Incubate the plate at 37°C, 5% CO₂ for at least 2 hours or until cells are ready for plating.
13. After the laminin incubation, pipet out most of the supernatant, but not all, from each coverslip or well using a P1000 pipettor.
14. Rinse with 500 μ l PBS.
15. Pipet out most of the PBS, but not all, from each well using a P1000 pipettor.
16. Repeat Steps 14-15
17. Add 300 μ l Medium N1(AP) to each well or coverslip using a P1000 pipettor.
18. Incubate the plate at 37°C, 5% CO₂ until cells are ready for plating.

*Ornithine and laminin can be substituted with Coating Materials B and C, respectively, from our Neuron Coating Solutions, Catalog Number: NCS.

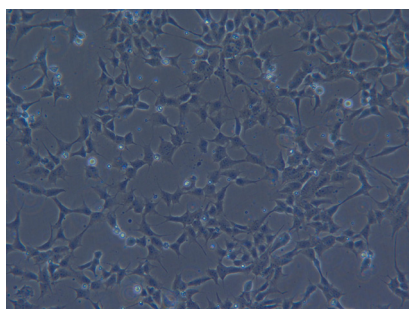
Passaging Cells

IMPORTANT! For the following steps, gently pipet and add solutions. Differentiating cells are delicate and should be handled with great care.

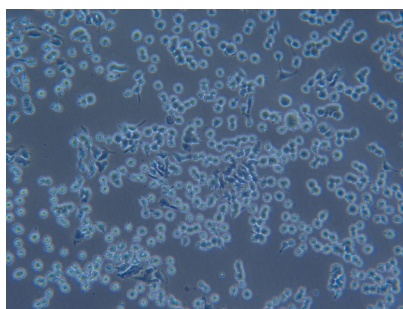
1. Make sure that Solution D1 is at room temperature for at least 1 hour before use.
2. Pipet out the old medium from each well using a P1000 pipettor and add 500 μ l PBS.
3. Pipet out the PBS from each well using a P1000 pipettor and add 80 μ l Solution D1.
4. Rock the plate 3 times to spread the Solution D1 evenly.
5. Incubate the cultures at 37°C, 5% CO₂ for 3 minutes.
6. Carefully pipet out Solution D1 from each well using a P200 pipettor and add 200 μ l Medium N1(AP).

IMPORTANT! Steps 7-9 below are critical. Perform these steps one well at a time. Refer to the images below to successfully manage cell treatment and dissociation.

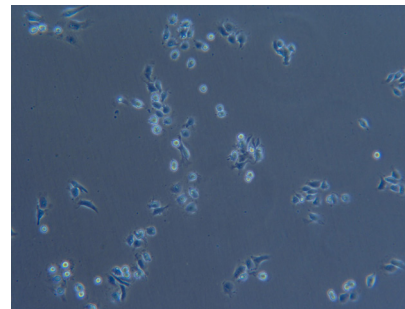
Before Solution D1 treatment



During Solution D1 treatment



After dissociation



-
7. Working one well at a time, disperse the medium quickly over the bottom surface of the well by pipetting 6-8 times to detach cells using a P200 pipettor.
 8. Observe cells and/or cell aggregates floating in the well under a microscope. It is normal that 10-20% of cells remain attached to the well bottom after pipetting. These clusters of cells are not supposed to be lifted. Do not attempt to detach all of the cells remaining on the well bottom.
 9. Gently pipet the cell suspension up and down in the well up to 5 times to break the cell aggregates using a P200 pipettor. Excessive pipetting can damage the already-suspended neuronal cells.
 10. Collect 200 μ l cell suspension from each well with a P200 pipettor set to 100 μ l and transfer all the cells to a new well or glass coverslip coated with ornithine and laminin 100 μ l at a time.
 - **IMPORTANT!** If cultures are >70% confluent on Day 4, transfer only half of the cell suspension (100 μ l) to each new well or glass coverslip to avoid excessively high cell density. Add 100 μ l Medium N1(AP) after the cell suspension is transferred to bring the total volume up to 200 μ l.
 - When transferring the cell suspension to a coverslip, bring the tip very close to the coverslip and pipet slowly to mount cells right at the center of the coverslip.
 11. Incubate the cultures at 37°C, 5% CO₂ for 1 hour.
 12. Observe each well under the microscope to confirm that the cells are attached to the well or glass coverslip.
 13. Incubate the cultures at 37°C, 5% CO₂ overnight.

Maintenance (Unpassaged Cultures Only)

1. Pipet out the old medium from each unpassaged well using a P1000 pipettor and add 500 μ l Medium N1(AP).
 - **IMPORTANT!** For the wells that are not passaged, the attachment of cells is weaker and so a small volume of the old medium (i.e., just enough to cover the surface of the well) should be left. The fresh medium should be applied along the wall of the well very slowly.
2. Incubate the cultures at 37°C, 5% CO₂ overnight.

Day 4



Maintenance

1. Warm Medium N1(AP) at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor and add 800 μ l Medium N1(AP).
 - **IMPORTANT!** For the wells that are not passaged, the attachment of cells is weaker and so a small volume of the old medium (i.e., just enough to cover the surface of the well) should be left. The fresh medium should be applied along the wall of the well very slowly.
3. Incubate the cultures at 37°C, 5% CO₂ for 2 days.

Day 6

Assay or Continuous Maturation

Differentiated neurons can be observed on Day 4. For more mature neurons, we recommend culturing cells until Day 6. From Day 6, users may maintain differentiated neurons in the maintenance medium best suited for their needs, though we recommend Quick-Neuron™ Cholinergic - Maintenance Medium, Catalog Number: CH-MM. Differentiation into cholinergic neurons after using the Quick-Neuron™ Cholinergic - SeV Kit can be confirmed with anti-TUBB3 (tubulin beta 3 class III, a global marker for neurons) and anti-ChAT (choline acetyltransferase, a cholinergic neuron marker) antibodies.

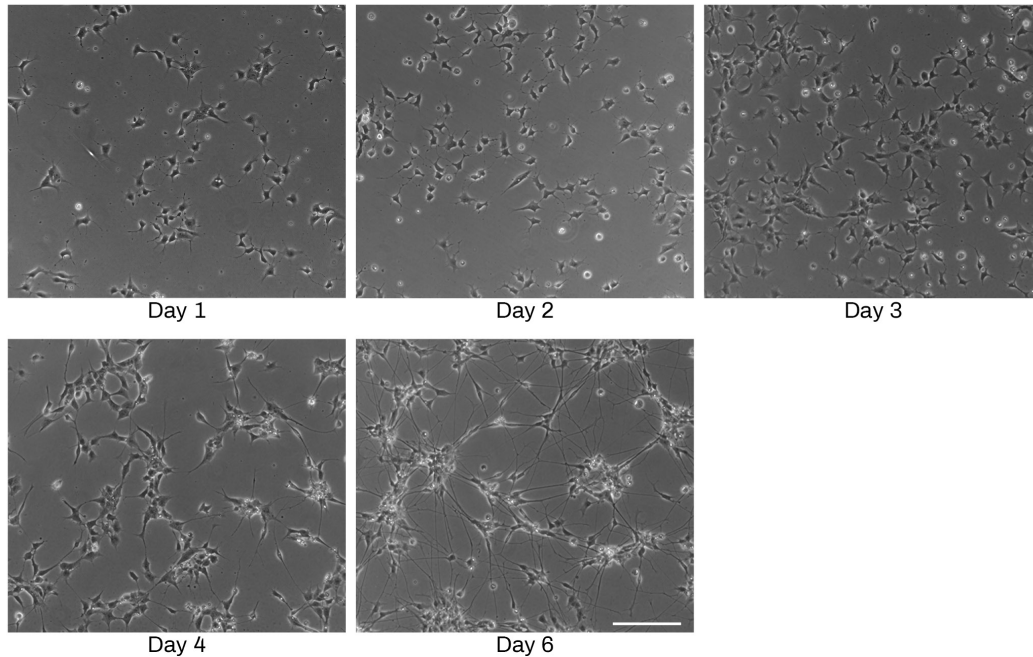


Figure 1. Representative images of Quick-Neuron™ Cholinergic - SeV Kit cell cultures on days 1-6 post-differentiation (scale bar = 100 μ m).

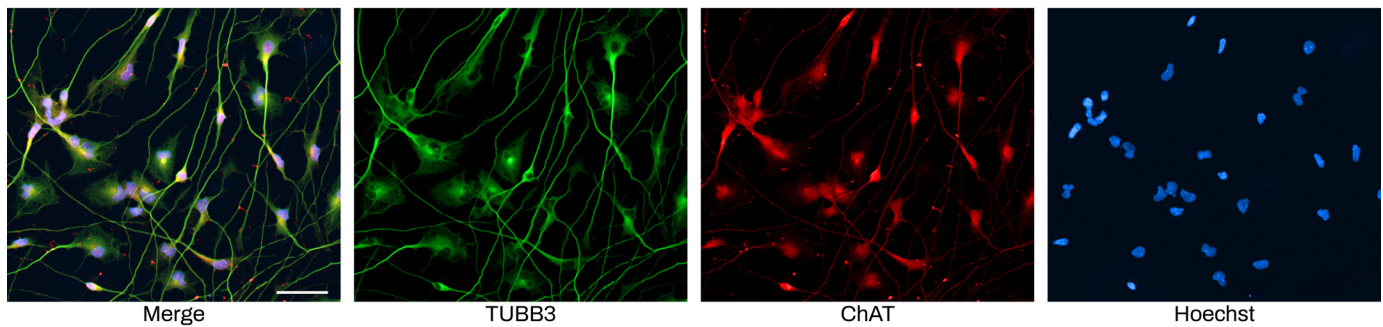


Figure 2. Immunofluorescent staining of Quick-Neuron™ Cholinergic - SeV Kit cell cultures shows typical neurite growth and expression of the pan-neuronal marker TUBB3 and the cholinergic neuron-specific marker ChAT on day 6 post-differentiation (scale bar = 100 μ m). Staining conditions: Anti- β -III tubulin monoclonal antibody (R&D Systems, Catalog Number: MAB1195, 1:250 dilution) in combination with a secondary antibody (Invitrogen, Catalog Number: A32723 Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, AlexaFluor Plus 488, 1:500 dilution). Anti-ChAT primary antibody (EMD Millipore, Catalog Number: AB143, 1:200 dilution) in combination with a secondary antibody (Invitrogen, Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, 1:500 dilution). Nuclei were counterstained with Hoechst 33324.