

TrailBio® A9 Dopaminergic Neurons

What are A9 Dopaminergic Neurons?

A9 dopaminergic neurons are a distinct subtype of midbrain neuron located in the *substantia nigra pars compacta* (SNc), where they play a key role in motor control. They are characterized by expression of the transcription factor SOX6, which distinguishes them from other dopaminergic subtypes such as A10 neurons, which are primarily involved in reward and motivation pathways. A9 neurons are particularly vulnerable in Parkinson's disease, making access to high-quality iPSC-derived A9 dopaminergic neurons valuable for disease modeling and evaluating cell-based therapeutic strategies both *in vitro* and *in vivo*.

How are these cells generated?

Our TrailBio® A9 Dopaminergic Neurons are differentiated from human iPSCs using a proprietary directed differentiation protocol that closely mimics natural development cues to generate cells with high purity and functional characteristics.

What applications are TrailBio® A9 Dopaminergic Neurons suitable for?

TrailBio[®] A9 Dopaminergic Neurons can be used in a range of applications, including modeling neurodegenerative disorders such as Parkinson's disease, investigating dopamine dysregulation, conducting neurotoxicity assays, advancing organ-on-a-chip systems, and supporting drug discovery efforts. Their selective vulnerability in Parkinson's disease makes them especially valuable for disease modeling and therapeutic development.

What markers do these cells express?

Our TrailBio[®] A9 Dopaminergic Neurons express tyrosine hydroxylase (TH), SLC6A3 (DAT), KCNJ6, NR4A2 (NURR1), and transcription factor SOX6 along with pan neuronal maturation markers such as MAP2 and SYN1. They do not express CALB1, a typical A10 marker.

What culture conditions are recommended for TrailBio® A9 Dopaminergic Neurons?

Cells should be maintained at 37 °C with 5% CO₂ in a humidified incubator during maturation. We recommend using the TrailBio[®] A9 Dopaminergic Medium as described in the User Instructions.

Are there further differentiation steps needed before these cells can be used in an assay?

TrailBio[®] A9 Dopaminergic Neurons require a 10-day maturation period post-thaw to reach full functionality. They are assay-ready between days 10 and 14, with day 10 providing reliable results and day 14 reaching higher maturity. While day 14 exhibits increased expression of certain markers of cell maturation, experiments conducted within this window (days 10–14) ensures reproducibility and consistency.

Are any genome modifications used in the production of these cells?

TrailBio[®] A9 Dopaminergic Neurons were produced without genome modifications. Their differentiation process closely mimics natural embryonic development using our proprietary HD-DoE[®] technology.

Do you provide custom differentiation services?

Yes, we offer custom differentiation and characterization services to meet specific research needs, including disease modeling and modified differentiation protocols. Please contact us at support@trailbio.com to plan your project.

How are cells and media shipped?

Cryopreserved cells are shipped in vials on dry ice under temperature-controlled conditions. Basal medium is shipped either on dry ice or on ice packs, and supplements are shipped on dry ice.

What is the recommended storage and shelf life?

Cryopreserved cells should be stored in liquid nitrogen (-135 °C to -195 °C) and used within 6 months from the date of purchase. Upon receipt, promptly transfer cell vials from dry ice to liquid nitrogen storage using proper PPE, avoiding any thawing until cells are ready to be used.

What are the recommended thawing procedures?

Thaw the vial of cryopreserved cells in a 37 $^{\circ}$ C water bath until just a small ice pellet remains. Transfer the contents to a 15 ml centrifuge tube and dilute dropwise with 5 ml of Basal Medium D. Centrifuge for 4 minutes at 200 \times g, then gently aspirate the supernatant and resuspend the pellet in 1 ml of Plating Medium. Perform a cell count using trypan blue. Each vial should contain over 3 million viable cells. Refer to the TrailBio® A9 Dopaminergic Neurons Kit User Instructions for a more detailed thawing and plating protocol.

What materials are required for thawing of the cells?

Required materials for cell thawing are a sterile 15 ml conical tube, pre-warmed medium, and sterile wide bore pipette tips.

What QC tests are performed on the iPSC-derived A9 dopaminergic neurons?

Cells are characterized by FACS for TH, TUBB3, and CD44 expression, by ICC for CALB1 (negative), and are screened for sterility and mycoplasma. Refer to the TrailBio[®] A9 Dopaminergic Neurons CoA for full details.

Does the cryopreservation medium contain DMSO?

Yes, the cryopreservation medium does contain DMSO, which is commonly used to protect cells during the freezing and thawing process.

How quickly are cells shipped and received?

Online orders are shipped within 7 business days with overnight delivery. We do not ship on Fridays, Saturdays or Sundays.

How can I use these cells?

Cells are for research use only, meaning no human, veterinary, therapeutic, or diagnostic use. You may use the cells for *in vitro* applications as well as *in vivo* pre-clinical research in animals. For more questions, please refer to our <u>Terms and Conditions | Trailhead Biosystems</u> or contact us at support@trailbio.com.

What support do you provide post-purchase?

We offer technical support, troubleshooting guidance, and access to detailed protocols to ensure successful cell culturing and experimental reproducibility. Please contact support@trailbio.com with any further questions.



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