TrailBio® A9 Dopaminergic Neurons Kit
User Instructions

Product Description

This protocol is designed to generate functional A9 dopaminergic neurons (DN) from a frozen vial of DN progenitors following 14 days of differentiation post-thaw.

Kit components (KEC05010301; kit with vial of 3M cells):

Component Name	Size	Quantity	Storage	Catalog Number
TrailBio® A9 Dopaminergic Neurons	1 ml	1 vial	LN2, Shipped on Dry Ice	EC050103010
TrailBio® Basal Medium D	60 ml	1 bottle	4 °C	BPS04006
TrailBio® Plating Supplement E (blue cap)	300 µl	1 vial	-20 °C	PPS05030
TrailBio® A9 Dopaminergic Medium Supplement (green cap)	800 µl	4 vials	-20 °C	DEC05401

Note: *Store components individually at the recommended storage conditions. Make complete medium in smaller batches as per "Differentiation medium preparation" below. Use cells within 6 months of date of purchase. Use medium and supplements within 3 months of date of purchase.*

Materials required but not included:

Name	Vendor	Catalog Number
Full-length human recombinant laminin-521	BioLamina	LN521-02
DPBS with Ca^{2+} and Mg^{2+}	Various	Various
TC-treated cell culture plate	Various	Various
Sterile 50 ml tubes	Various	Various
Sterile 15 ml centrifuge tubes	Various	Various

Preparation of Solution and Medium:

Note: *Use proper sterile culture techniques throughout all coating, preparation and culture steps.*

Coating of culture plate:

Follow manufacturers' instructions for coating culture vessels. Briefly, see below:

1. Thaw laminin-521 at 4 °C as per manufacturer's instructions.
2. To make coating solution, dilute laminin-521 in DPBS with Ca^{2+}/Mg^{2+} to reach 5 µg/ml concentration.
3. Distribute the solution evenly in each well at 0.78 µg/cm^2 (e.g. 50 µl in a 96-well plate).
4. Parafilm wrap the culture vessel and incubate it overnight at 4 °C.

   Note: *Coated plates may be stored for 1 week at 4 °C.*
5. Bring coated cell culture vessel to room temperature prior to use.

   Note: *It is not recommended to leave coated plates at room temperature for longer than 45 minutes.*

Plating Medium preparation:

1. Thaw the vial of Plating Supplement E (blue cap) at room temperature or overnight at 4 °C.
2. Briefly pulse vortex thawed Plating Supplement E (blue cap) for 3-4 seconds to ensure a homogenous mixture.

Note: *After vortexing, ensure all supplement contents are collected at the bottom of the tube by briefly centrifuging for 3 seconds or tapping the tube.*

3. Add 300 µl of Plating Supplement E (blue cap) to 2.7 ml Basal Medium D Pipette up and down 2-3 times avoiding bubble formation, to ensure it is thoroughly mixed.

Note: *Plating medium should be prepared immediately before use and should be at room temperature prior to use. Repeated freeze/thaw cycles of medium and supplements are not recommended.*

Differentiation Medium preparation:

1. Thaw a vial of A9 Dopaminergic Medium Supplement (green cap) at room temperature or overnight at 4 °C.
2. Briefly pulse vortex thawed A9 Dopaminergic Medium Supplement (green cap) for 3-4 seconds to ensure a homogenous mixture.

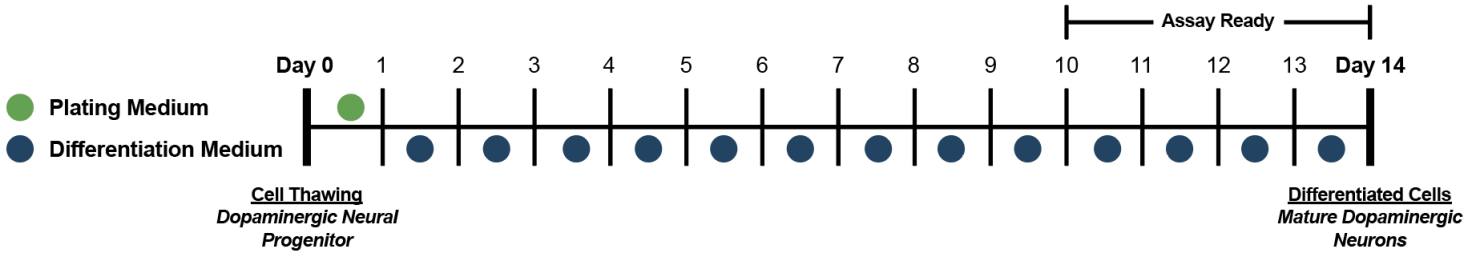
Note: *After vortexing, ensure all supplement contents are collected at the bottom of the tube by briefly centrifuging for 3 seconds or tapping the tube.*

3. Add 800 µl A9 Dopaminergic Medium Supplement (green cap) to 11.2 ml of Basal Medium D. Pipette up and down 2-3 times avoiding bubble formation, to ensure it is thoroughly mixed.

Note: *Prepared Differentiation Medium can be stored at 4 °C for up to 72 hours and should be at room temperature prior to use. Repeated freeze/thaw cycles of medium and supplements are not recommended.*

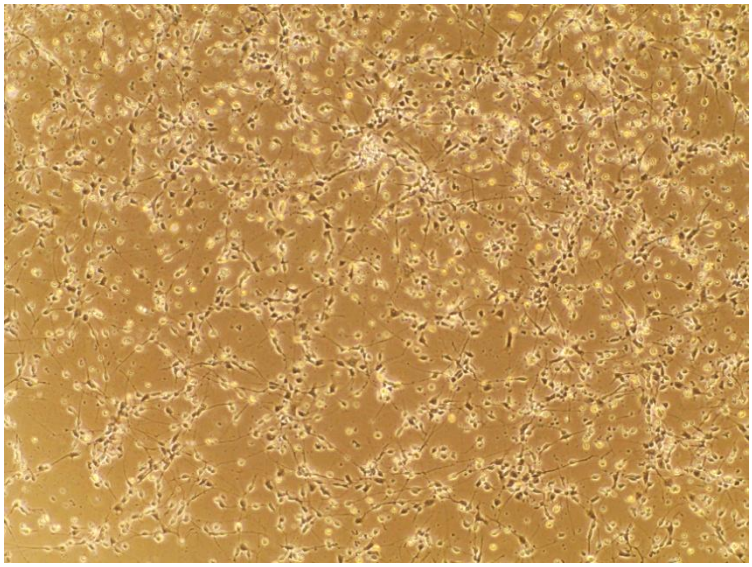
Directions for Use:

Summary of Differentiation Protocol:



Thawing and Plating of Cells (Day 0):

1. Bring coated plate to room temperature. (see above; “Coating of culture plate”).
   Note: *Ensure coating material has not dried in wells, if so, avoid using those wells.*
2. Prepare Plating Medium as per the “Plating Medium preparation” step above.
   Note: *Allow Plating Medium to reach room temperature before use.*
3. Swirl the vial of cryopreserved cells in a 37 °C water bath until the cell mixture is nearly but not completely thawed, leaving a small ice pellet in the vial.
4. Transfer contents of the vial to a 15 ml centrifuge tube and dilute the cell mixture dropwise with 5 ml of Basal Medium D.
5. Centrifuge the cell mixture for 4 minutes at 200 x g.
6. Aspirate the supernatant gently and resuspend the cell pellet in 1 ml of Plating Medium.
7. Take a sample of the cell mixture to perform a cell count. Each vial should contain ≥ 3 million viable cells as per trypan blue staining.
   Note: *You may have an excess of cells for this protocol.*
8. Add Plating Medium to the cells to achieve a target density of 250k cells/cm^2.
9. Prior to plating cells, remove the remaining coating solution from the coated plate.
   Note: *Add cells promptly after removing the coating to prevent wells from drying.*
10. Plate cells onto coated plates and incubate at 37 °C.



TrailBio® A9 Dopaminergic Neuron Cells
1 day post-thaw

Differentiation (Day 1, onward):

1. Prepare Differentiation Medium as per the “Differentiation Medium preparation” step on Page 2.
2. Tilt plate and gently hand-aspirate the Plating Medium from the wells, rinse gently with DPBS, and add Differentiation Medium. For a 96-well plate, use 100 µl per well.

Note: *Non-viable cells are expected in the culture and will be removed during media exchange.*

3. Change medium every 48 hours for a total of 13 days, preparing additional Differentiation Medium as needed.

Note: *If after 24 hours the medium is spent with a yellow / orange color, perform a media change.*

Note: *Neurons in adherent culture may show signs of detachment during the culture process. It is recommended that careful hand aspiration be performed during media exchange. If the culture shows signs of cell detachment, a half media change may be performed instead of a full media change to reduce the risk of further cell detachment.*

4. From days 10-14, cells may be fixed or used for downstream applications.

Note: *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 the middle of this window (days 10–14) ensure reproducibility and consistency.*

Note: *TrailBio® A9 Dopaminergic Neurons may have a small population of glial cells (CD44+) that improves attachment and survivability of neurons long-term. It is normal to see a small amount of proliferation of these cells throughout the culture. The percentage of CD44+ cells may fluctuate between lots.*