axol

Cortical Neural Induction Kit

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Product Information

Catalog. No.	Product Name	Format	Stock Conc.	Storage on Arrival	Thawing Instructions	Storage Once Thawed
ax0001	Cortical Neural Induction Kit – Medium A	125 mL	1x	Aliquot & store at -80ºC for up to 6 months	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week
	Cortical Neural Induction Kit – Medium B	125 mL	1x	Aliquot & store at -80ºC for up to 6 months	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week
	Unlock (ax0044)	25 mL	1x	Aliquot & store at -80ºC for up to 6 months	Overnight at 4ºC	Once thawed store aliquot at 4ºC for up to 1 week

Additional Reagents		
Product Name	**Provider**	**Catalog. No.**
Essential 8™ Medium	Thermo Fisher Scientific	A1517001
Y-27632 2HCl (ROCK inhibitor)	Selleck Chemicals	S1049
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher Scientific	A14700

The Cortical Neural Induction Kit volumes are sufficient for induction of two T25 flasks of pluripotent stem cells.

Important!

Axol Cortical Neural Induction Media DO NOT contain antibiotics or antifungal agents. Axol Bioscience does not recommend the use of antimicrobial agents such as penicillin, streptomycin and amphotericin. Antimicrobial agents should not be necessary if proper aseptic technique is adopted.

Preparation of Reagents

Cortical Neural Induction Kit - Medium A

- Upon receipt, aliquot and store **Medium A** at **-80°C** protected from light.
- When ready to use, thaw an aliquot of **Medium A** overnight at **4°C** in the dark.
- Avoid multiple freeze-thaw cycles.
- Minimize the exposure to light.

Cortical Neural Induction Kit - Medium B

- Upon receipt, aliquot and store **Medium B** at **-80°C** protected from light.
- When ready to use, thaw an aliquot of **Medium B** overnight at **4°C** in the dark.
- Avoid multiple freeze-thaw cycles.
- Minimize the exposure to light.

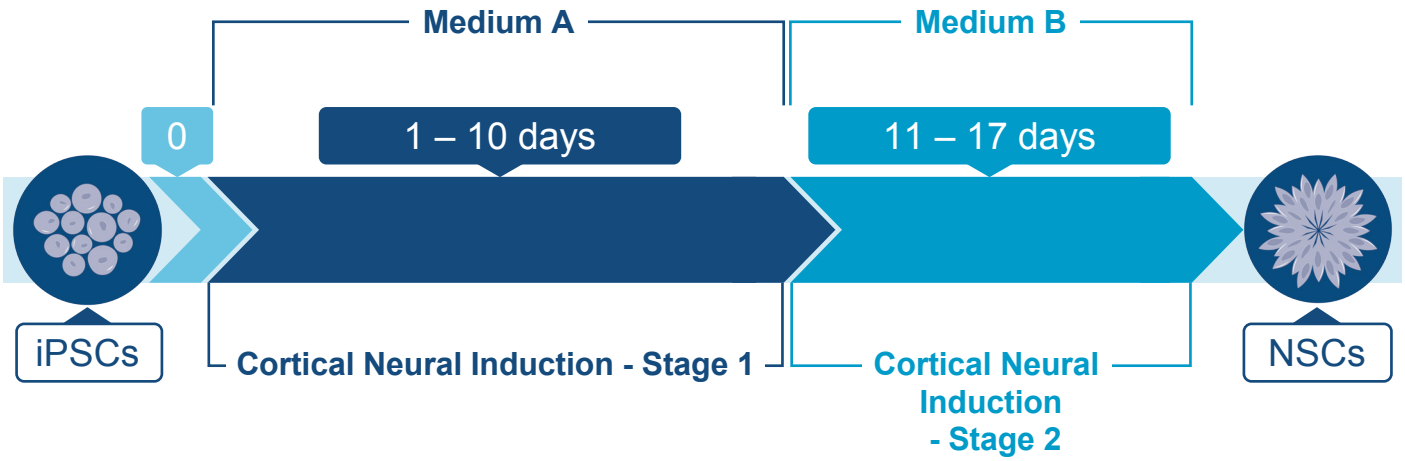
Cortical Neural Induction Kit - Coating Reagent

- Upon receipt, aliquot and store **Vitronectin (VTN-N)** following the supplier's instructions.
- Axol recommend to dilute the **Vitronectin (VTN-N)** **1:50** in Dulbecco's-PBS (1x) (D-PBS without calcium or magnesium) to make a 1x working solution (e.g. **120 µL** **Vitronectin (VTN-N)** in **6 mL** of D-PBS).
- **DO NOT VORTEX** the **Vitronectin (VTN-N)**.
- Pre-coat culture vessels with the working solution of **Vitronectin (VTN-N)**. We recommend coating a T25 flask with **6 mL** of 1x working solution.
- Incubate at **37ºC**, **overnight**.

Unlock

- Upon receipt aliquot and store **Unlock** at **-80°C** protected from light. Stored at **-80°C** the reagent is stable for 6 months from date of shipment.

Protocol Overview



Seeding iPSCs

- The iPSCs should be pre-cultured in the recommended culture system for the cells such that they are healthy and proliferative prior to proceeding with this protocol.
- In preparation for seeding the iPSCs, prepare the required number of T25 flasks by coating with the 1x working solution of **Vitronectin (VTN-N)** **overnight** at **37°C**.
- When the iPSCs have reached **80% confluency**, they are ready to be passaged.
- Remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with D-PBS. We recommend using **2 mL per 10 cm²** of culture surface area. Discard the D-PBS.
- Add **1 mL per 10 cm²** of culture surface area of **cold/room temperature** **Unlock** passaging reagent. Evenly distribute it over the entire cell layer. Incubate the cells for **5 minutes** at **37°C**. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached, transfer the cells drop-wise into a 15 mL sterile conical tube. Gently add four volumes of **room temperature** **Essential 8 Medium**. (For example if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge cells at **200 x *g*** for **5 minutes** at **room temperature**.
- Gently resuspend the cell pellet in **1-2 mL** of **room temperature** **Essential 8 Medium** + **10 µM** **Y-27632 2HCl** and perform a cell count and cell viability quantification.

Important!

The iPSCs should be healthy and proliferating prior to neural induction. Cell viability should be greater than 75%.

- Dilute the cell suspension in the required volume of **room temperature Essential 8 Medium** + **10 µM Y-27632 2HCl**.
- Seed the iPSCs into the **pre-coated** T25 flasks at a seeding density of **250,000 cells/cm²**. The day of seeding is **Day 0**.
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.

Cortical Neural Induction

Stage 1

- On **Day 1**, the iPSCs should be 80-90% confluent and ready for neural induction. Gently replace the cell culture medium with **room temperature Cortical Neural Induction Kit – Medium A** (**Medium A; without Y-27632 2HCl**).
- Between **Days 2 to 10**, gently replace the culture medium **daily** with fresh, **room temperature Medium A**.
- In preparation for passaging, thaw an aliquot of **Vitronectin (VTN-N)** at **4°C** on **Day 10**.
- Coat the required number of T25 flasks with the 1x working solution of **Vitronectin (VTN-N)** **overnight** at **37°C**.
- On **Day 11**, the cells should be passaged.

Stage 2

- On **Day 11**, remove all spent medium from cell culture vessels.
- Gently rinse the surface of the cell layer once with D-PBS. We recommend using **2 mL per 10 cm²** of culture surface area. Discard the D-PBS.
- Add **1 mL per 10 cm²** of culture surface area of **cold/room temperature Unlock** passaging reagent. Evenly distribute it over the entire cell layer. Incubate the cells for **5 minutes** at **37°C**. Observe the cells at regular intervals for detachment from the culture vessel.
- Once the cells have detached, transfer the cells drop-wise into a 15 mL sterile conical tube. Gently add four volumes of **room temperature Cortical Neural Induction Kit – Medium B** (**Medium B**). (For example if 1 mL of **Unlock** is used, then add 4 mL of the medium to stop the reaction). Gently pipette up and down a few times to disperse the medium.
- Centrifuge cells at **200 x *g*** for **5 minutes** at room temperature.
- Gently resuspend the cell pellet in **1-2 mL** of **room temperature Medium B** + **10 µM Y-27632 2HCl**.
- Dilute the cell suspension in the required volume of **room temperature Medium B** + **10 µM Y-27632 2HCl**.
- Perform a **1:1 passage** into the **pre-coated** T25 flasks (transfer all of the dissociated cells from 1 flask into 1 new flask).
- Incubate the cells at **37°C**, **5% CO_2** in a humidified incubator.
- On **Day 12**, gently replace the culture medium with fresh, **room temperature Medium B** (**without Y-27632 2HCl**).
- On **Day 14** and **Day 16**, gently replace the culture medium with fresh, **room temperature Medium B** (**without Y-27632 2HCl**).
- On **Day 17**, the induction process is complete and there should be a pure population of neural stem cells.
- Neural rosettes may be visible under the microscope at this stage, although this property can vary between iPSC lines.
- Proceed with experiment assays by passaging the cells with **Unlock** into the required culture vessels.

Neural Stem Cell Culture Systems

- We strongly recommend that **Axol Neural Stem Cell Media & Reagent Bundles** (sold separately) are used for subsequent neural stem cell expansion and differentiation. Each bundle contains the necessary media and reagents required for your preferred culture system.

Synchronous Differentiation

Pure population of cerebral cortical neurons

Culture large populations of neurons for use across several assays

Achieve a pure population of neurons in less time

Spontaneous Differentiation

Mixed population of neuronal and glial cells

Get large populations of neuronal & glial cells for use across multiple experiments

Go directly into a mixed population of neuronal & glial cells

Catalog. No.	Product Name	Product Description
ax0101	Neural Stem Cell Media & Reagent Bundle - Expansion and Synchronous Differentiation (System A)	Contains all of the neural media and reagents required to expand and synchronously differentiate Axol neural stem cells and maintain the differentiated neurons
ax0102	Neural Stem Cell Media & Reagent Bundle - Synchronous Differentiation (System B)	Contains all of the neural media and reagents required to synchronously differentiate Axol neural stem cells and maintain the differentiated neurons
ax0103	Neural Stem Cell Media & Reagent Bundle - Expansion and Spontaneous Differentiation (System C)	Contains all of the neural media and reagents required to expand and spontaneously differentiate Axol neural stem cells and maintain the differentiated neural cells
ax0104	Neural Stem Cell Media & Reagent Bundle - Spontaneous Differentiation (System D)	Contains all of the neural media and reagents required to spontaneously differentiate Axol neural stem cells and maintain the differentiated neural cells

Troubleshooting

- **I'm not culturing my iPSCs in Essential 8™ Medium. Can I use this induction kit?**
  Yes, the kit has also been validated to work with iPSCs cultured in mTeSR™1 medium (STEMCELL Technologies). For other culture media, we would recommend setting up a back-up flask of iPSCs in the existing culture medium before transitioning directly into Cortical Neural Induction Kit - Medium A.
- **My iPSCs are not 80-90% confluent on Day 1. What should I do?**
  If the cells are <80% confluent, wait until they have reached the required confluency before proceeding with neural induction. If the cells are fully confluent, proceed with neural induction immediately.
- **My cells look unhealthy during induction. What should I do?**
  Ensure that you perform cell viability quantification during the passaging steps to determine the percentage of viable cells. Cell viability should be greater than 75% throughout the procedure. Ensure that 10 µM Y-27632 2HCl is added following passaging since this will increase cell viability and attachment. If there has been a large amount of cell loss, the cells can be re-plated at a higher seeding density. If the iPSCs were not cultured in Essential 8™ Medium prior to starting neural induction, it may be necessary to transition the iPSCs into Essential 8™ Medium.
- **My cells are starting to peel during days 2-10. What should I do?**
  Most iPSC lines should not peel during neural induction although this can happen with less robust lines. A small amount of peeling at the edges of the monolayer is not a problem. If the cell layer peels off early in induction, the induction process will need to be repeated by re-plating iPSCs. Ensure that 10 µM Y-27632 2HCl is added for the first 24 hours after plating. If the cell layer starts to peel off after Day 9, proceed with passaging the cells into Cortical Neural Induction Kit - Medium B immediately.
- **How do I know if the induction has been successful?**
  Neural rosette formation may be visible by Day 16. As the cells transition from iPSCs to neural stem cells, the cell morphology should change. Neural stem cells are typically smaller than iPSCs. The expression of neural stem cell markers such as nestin, PAX6 and FOXG1 can also be assessed. The absence of iPSC markers such as NANOG and OCT4 can also be assessed.

Usage Statement

Our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans.

Got any questions? Need help with the protocol?
Contact Axol Technical Support at **support@axolbio.com**
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Notes

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