Transfection of Axol™ Human Neural Progenitor Cells (hNPCs) using Axol ReadyFect™

Catalog No. ax0051

Application Protocol

Version 2.0

axol

Table of Contents

Product Information

Catalog no.	Product Name:	Format	Storage upon Arrival:	Thawing Instructions:
ax0013 - ax0016	Axol hNPCs	2M viable cells per cryovial	Liquid Nitrogen	Follow our protocol
ax0111 - ax0115	Axol AD hNPCs	2M viable cells per cryovial	Liquid Nitrogen	Follow our protocol
ax0031	Axol Neural Maintenance Medium Kit includes ax0031a supplement/ ax0031b basal medium)	1 x 7.5 mL 1 x 500 mL	-80ºC and 4-8ºC	Follow our protocol for medium preparation
ax0051	Axol ReadyFect™	1 x40 µL	-20ºC	Thaw immediately before use. Avoid repeated freeze-thaw cycles

Note: Catalog no. **ax0051** includes 1 tube containing 40 µL of Axol ReadyFect™. According to our guidelines, this provides enough Axol ReadyFect™ to transfect approximately 60% confluent Axol hNPCs cultured in:

1 x 96-well plate, 1 x 24-well plate, 1 x 6-well plate, 4 x 60 mm dishes or 1 x T-75 flask

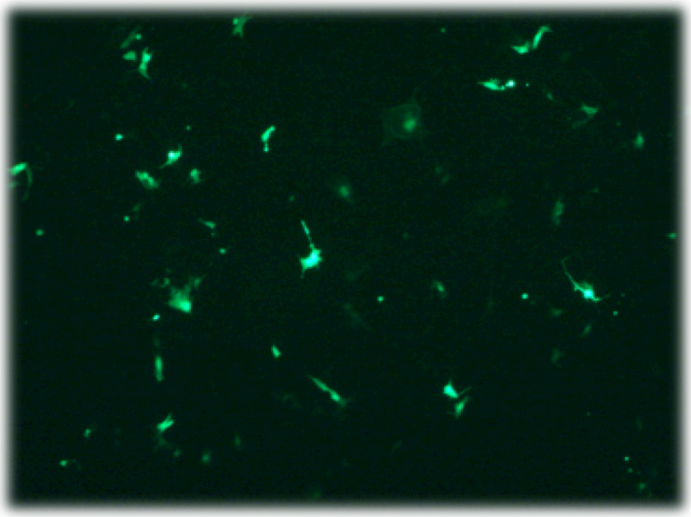
Axol ReadyFect™ Technology Overview

Axol ReadyFect™ is based on the Tee-Technology (“Triggered Endosomal Escape”) that combines and exploits the properties of cationic lipids and polymers to achieve an extremely efficient DNA delivery into Axol Human Neural Progenitor Cells (hNPCs).

Axol ReadyFect™ is a powerful reagent optimised to transfect Axol hNPCs with improved cytoplasmic release and better biodegradability without impacting phenotype and differentiation potential.

The instructions given below have been validated on Axol hNPCs. We recommend to use **3 µL of Axol ReadyFect™ / 1 µg of DNA**. You can use your complete Axol Neural Maintenance medium, except during the preparation of the **Axol ReadyFect™** / DNA complexes where you should use PBS.

Nucleic acids should be as pure as possible. Use transfection grade plasmid DNA (high degree of supercoiled forms) to achieve high expression. Avoid long incubation time of the DNA solution in PBS before the addition of **Axol ReadyFect™** to circumvent any degradation or surface adsorption.



Axol ReadyFect™ leads to 30-40% transfection efficiency. The image demonstrates transfection results using 3 µL of Axol ReadyFect™ / 1 µg of a GFP-encoding vector (pVectOZ-GFP). GFP expression was evaluated 2 days post-transfection.

Considerations Before You Begin

1. Thaw and culture your Axol hNPCs or Axol AD hNPCs according to our instruction manuals, which can be found at www.axolbio.com/instruction-manuals.

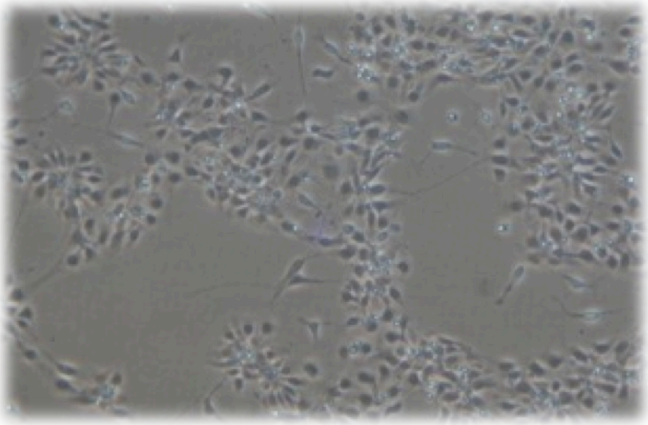
2. Ensure that the cells are **50-70% confluent** at the point of transfection.

3. DNA and Axol ReadyFect™ solutions should be at **room temperature** and be gently vortexed prior to use.

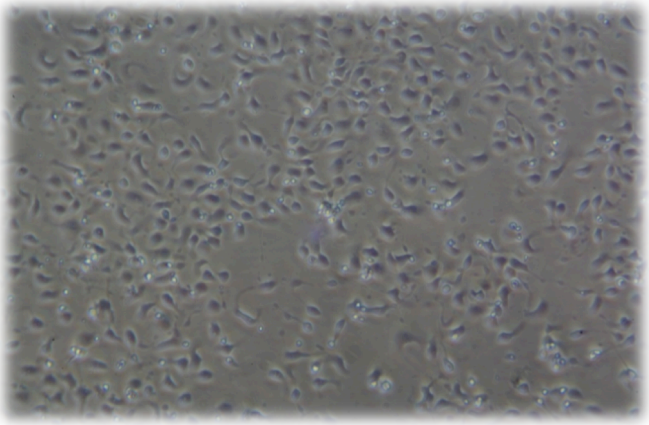
4. Calculate the amount of ReadyFect™ that you will require. Use **3 µL of Axol ReadyFect™ per µg** of DNA, as this is the optimal ratio (3:1) for Axol hNPCs. However, depending on your experimental requirements, some optimization may be required. In this case, please follow suggestions in the Optimization section of this manual.

Warning: Do not let your hNPC culture exceed 70% confluence as this will reduce your transfection efficiency. In the representative images below, 50-60% confluence is ideal whereas 80% confluence is too high.

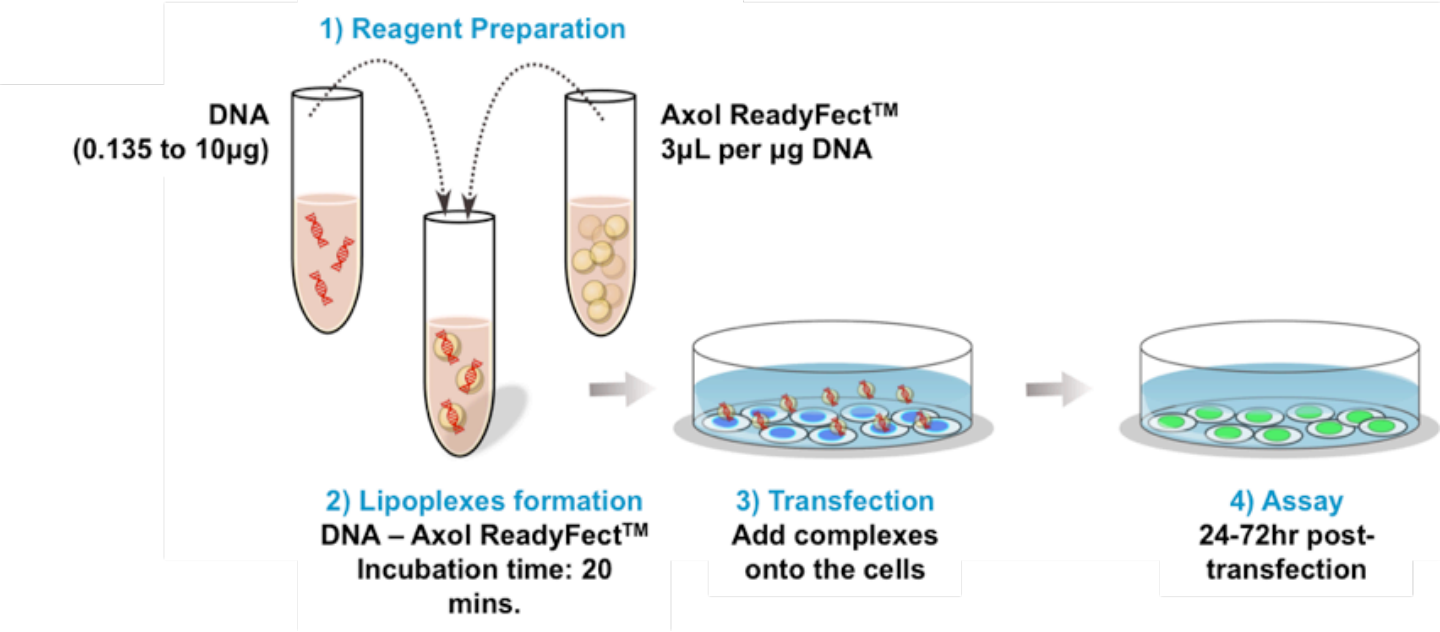
50 - 60% Confluence



80% Confluence



Standard Transfection Protocol



Schematic Overview of The Standard Transfection Protocol

1. Prepare your DNA solution by diluting 0.125 µg to 10 µg of DNA in 25 µL to 350 µL of PBS according to guidelines in **Table 1**.

2. Prepare your Axol ReadyFect™ solution by 0.375 µL to 30 µL of Axol ReadyFect™ in PBS according to guidelines in **Table 1**.

3. Add DNA solution to the Axol ReadyFect™ solution, mix gently by vortexing slowly or pipetting up and down 4-5 times.

4. Incubate the mixture for **20 mins** at **room temperature.**

5. Add the mixture to Axol hNPCs (growing in Complete Axol Neural Maintenance Medium) dropwise and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

6. Incubate the cells at **37°C, 5% CO_2**.

7. Evaluate your transgene expression **24 to 72 hrs** post-transfection.

Cell Culture Dish	DNA Quantity	Dilution Volume	Axol ReadyFect™ Volume	Dilution Volume	Final Transfection Volume
96 well plate	0.125 µg	25 µL	0.375 µL	25 µL	200 µL
24 well plate	0.5 µg	50 µL	1.5 µL	50 µL	500 µL
6 well plate	2 µg	100 µL	6 µL	100 µL	2 mL
60 mm dish	3 µg	150 µL	9 µL	150 µL	4 mL
T-75 flask	10 µg	350 µL	30 µL	350 µL	10 mL

Table 1: Suggested amounts of DNA and reagent depending on your cell culture format

Optimisation Steps

Although high transfection efficiencies can be achieved with the standard protocol, some optimization might be necessary in order to obtain optimal transfection efficiency.

Several parameters can be optimized including ratio of Axol ReadyFect™ to DNA, quantity of DNA used, cell density and incubation time.

Top Tip: Modify one parameter at a time while keeping the other parameters (cell number, incubation time etc.) constant. The two most critical variables are the ratio of Axol ReadyFect™ to DNA and the quantity of DNA.

1. **Axol ReadyFect™ / DNA ratio:** This is the most important parameter for optimization. First, maintain a fixed quantity of DNA (according to the size of your culture dish or cell number) and then vary the amount of Axol ReadyFect™ reagent as indicated in **Table 2**. **You can test ratios from 1 to 6 µL of Axol ReadyFect™ reagent per 1 µg DNA**.

Culture Vessel	DNA Quantity	Axol ReadyFect™ Volume
96 well plate	0.125 µg	0.1 – 0.7 µL
24 well plate	0.5 µg	0.5 – 3 µL
6 well plate	2 µg	2 – 12 µL
60 mm dish	3 µg	3 – 18 µL
T-75 flask	10 µg	10 – 60 µL

Table 2: Suggested range of Axol ReadyFect™ for optimisation.

2. **Quantity of DNA:** After optimization of the Axol ReadyFect™ / DNA ratio, proceed to adjust the amount of DNA required by maintaining a fixed ratio of Axol ReadyFect™ to DNA, and varying the DNA quantity over the suggested range (**Table 3**).

Culture Vessel	DNA Quantity	Transfection Volume
96 well plate	0.1 – 0.5 µg	200 µL
24 well plate	0.25 – 2 µg	500 µL
6 well plate	1 – 4 µg	1 mL
60 mm dish	5 – 30 µg	4 mL
T-75 flask	15 – 90 µg	10 mL

Table 3: Suggested range of DNA amounts for optimisation

3. **Axol ReadyFect™:** Several tests demonstrated that the use of PBS to prepare the DNA and Axol ReadyFect™ solutions leads to more reproducible transfections and in some cases higher efficiency, particularly with lower volumes of transfection reagent. PBS composition: 137mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4 and 6.5mM Na_2HPO_4 x 2 H_2O; pH7.4.

4. **Transfection Volume:** To increase the efficiency of transfection you can reduce the transfection volume.

Quality Control

To assure the performance of each Axol ReadyFect™ lot, we control the quality of each component using rigorous standard procedures. The following *in vitro* assays are performed to guarantee the function, quality and activity of each component.

Specification	Standard Quality Controls
Purity	Silica Gel TLC assays. Every compound shall have a single spot.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Transfection efficacies on MSC cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1. **Axol ReadyFect™ / DNA ratio.** Optimize the reagent / DNA ratio by using a fixed amount of DNA (µg) and vary the amount of reagent from 2 times less up to three times more than the suggested amount detailed in the Table 2. 2. **DNA.** Vary the DNA amount with the recommended or optimized Axol ReadyFect™ / DNA ratio. 3. **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal cell confluency should range from 50 to 70%. 4. **DNA quality.** Nucleic acids should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies**.** Use nuclease-free materials. 5. **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. 6. **Medium used for preparing DNA / transfection reagent complexes**. It is critical that serum-free medium or buffer (HBS, PBS) is used during the preparation of the complexes. Avoid any direct contact of pure Axol ReadyFect™ and pure nucleic acid solution with the plastic surface. 7. **Old Axol ReadyFect™ / DNA complexes.** The Axol ReadyFect™ / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hr can aggregate. 8. **Transgene detection assay**. Ensure that your post-transfection assay is properly set up and includes a positive control. 9. **Transfection reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use.
Cellular toxicity	1. **Transgene product is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a DNA control. 2. **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant- and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 3. **Concentration of Axol ReadyFect™ / nucleic acid too high.** Decrease the amount of DNA / Axol ReadyFect™ complexes added to the cells by lowering the DNA or the Axol ReadyFect™ concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 4. **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4hr.

Technical Support

- ## Online Resources

  Please visit our website at www.axolbio.com for additional product information and *Technical Resources*, including instruction manuals, application protocols, video guides, wall charts and webinars.

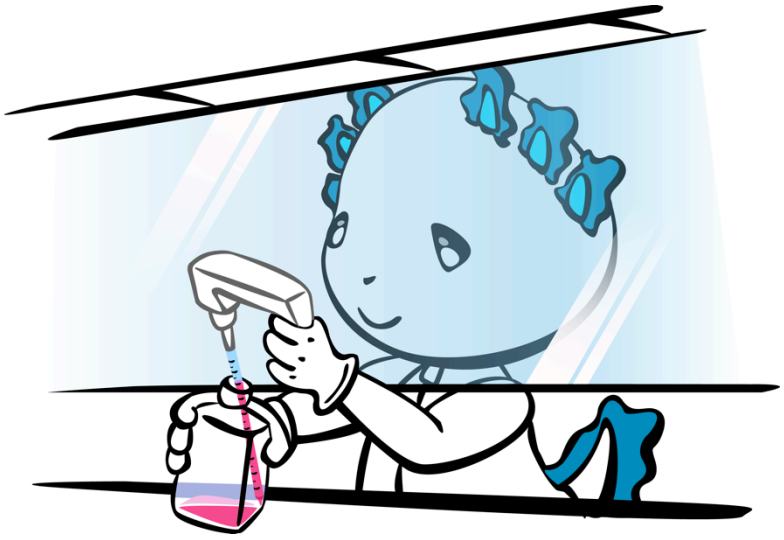
- ## Contact Us

  For more information or technical assistance, call +44 (0) 1223 497 119, or email support@axolbio.com. US Toll Free Tel: 1-800-678-2965 (1-800-678-AXOL), US Toll Free Fax: 1-800-861-2965 (1-800-861-AXOL).

- ## Certificate of Analysis

  The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website.

  Go to www.axolbio.com/certificate-of-analysis-lookup and search for the Certificate of Analysis with product lot number, which is printed on the cryovial label.



Don’t forget to rate, review and register your Axol product at www.axolbio.com