GeneXPlus Transfection of Plasmid DNA into SH-SY5Y Cells

SH-SY5Y (ATCC® No. CRL-2266™) is a human neuroblastoma cell line that was derived from a bone marrow biopsy. ATCC achieved transfection efficiencies of approximately 40% using the protocol described below.

General Considerations for using the GeneXPlus transfection reagent:

- **All steps should be performed in a biosafety cabinet using proper aseptic technique.**
- **Cell conditions.** Cells should be passaged at least once after thaw and the use of low-passage cells is recommended. Passage the cells 18-24 hours before transfection to ensure the cells are actively dividing and that they will be at the appropriate cell density at the time of transfection. Make sure that the cells are healthy and are ≥ 90% viable, prior to transfection.
- **Seeding density.** Cell density should be 50-80% confluent on the day of transfection. See specified seeding density in the individual protocols and in Table 1. *Note: Determine the optimal cell density for each cell type in order to maximize transfection efficiency.*
- **DNA purity.** Use highly purified plasmid preps that are free from phenol or other contaminants. Plasmid DNA preps that are endotoxin-free are desirable.
- **Presence of antibiotics and other inhibitors.** Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing serum and low levels of antibiotics if required.
- **Complex formation conditions.** Prepare GeneXPlus reagent and DNA complexes in serum-free growth medium. ATCC recommends using Opti-MEM I Reduced-Serum Medium to dilute the DNA before complex formation.

Materials required:

<table>
<thead>
<tr>
<th>Material Required</th>
<th>Catalog No.</th>
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</thead>
<tbody>
<tr>
<td>SH-SY5Y cells</td>
<td>ATCC® CRL-2266™</td>
</tr>
<tr>
<td>Eagle’s Minimum Essential Medium</td>
<td>ATCC® 30-2003</td>
</tr>
<tr>
<td>FBS</td>
<td>ATCC® 30-2020</td>
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<tr>
<td>GeneXPlus</td>
<td>ATCC® ACS-4004</td>
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<tr>
<td>Opti-MEM® I Reduced-Serum Media</td>
<td>Life Technologies™ 31985-062</td>
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<tr>
<td>Plasmid DNA of interest (1µg/µL)</td>
<td></td>
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<tr>
<td>Tissue culture plates and supplies</td>
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</tbody>
</table>
GeneX<em>Plus</em> Transfection of Plasmid DNA into SH-SY5Y Cells

Protocol:
The following protocol describes how to transfect plasmid DNA into SH-SY5Y cells using the GeneX<em>Plus</em> Reagent in a **24 well plate**. The reaction can be scaled up as needed. Please refer to Table 1 for recommended reaction conditions for other dish or plate sizes.

A. Preparation of the cells for transfection
   - **The day before transfection:**
     1. Count and measure cells for density and viability.
     2. Plate $2.0 \times 10^5$ cells per well in 0.5 mL of complete growth medium. Cell density should be 50 - 80% confluent on the day of transfection.
     3. Incubate cells overnight at $37^\circ C$ with 5% CO$_2$.
   - **The day of transfection:**
     1. Remove old media.
     2. Replace old media with fresh complete growth media to a total volume of 0.5 mL.

B. Preparation of the DNA: TransfeX transfection complexes
   1. Warm GeneX<em>Plus</em>, plasmid DNA, and Opti-MEM I Reduced-Serum Medium to room temperature and vortex gently to mix.
   2. Pipette 50 µL Opti-MEM I Reduced-Serum Medium into a sterile microcentrifuge tube.
   3. Add 0.5 µL (1.0 µg/µL) plasmid DNA.
   4. Mix thoroughly with gently pipetting.
   5. Add 1.0 µL GeneX<em>Plus</em> Reagent to the diluted DNA mixture. **Note: Do not let the pipette tip or the reagent come into contact with the sides of the plastic tube.**
   6. Mix GeneX<em>Plus</em> complexes thoroughly using either a vortex or by pipetting briefly.
   7. Collect contents at bottom of the tube using a mini-centrifuge.

C. Addition of DNA: GeneX<em>Plus</em> transfection complexes to the cells
   1. Distribute the complexes to the cells by adding the complexes drop-wise to different areas of the wells.
   2. Gently rock the culture vessel back and forth and from side to side to evenly distribute the GeneX<em>Plus</em>:DNA complexes.

D. Post-Transfection Handling
   1. Incubate for **24-72** hours. Replace transfection medium with fresh complete growth medium every 24 hours post transfection.
   2. Wait for 18-24 hours post-transfection before assaying for transgene expression.
GeneXPlus

Transfection of Plasmid DNA into SH-SY5Y Cells

Table 1: Recommended Reaction Conditions for different size culture vessels.

<table>
<thead>
<tr>
<th>Culture Vessel</th>
<th>24 well plate</th>
<th>12 well plate</th>
<th>6 well plate</th>
<th>10 cm dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area</td>
<td>1.9 cm²</td>
<td>3.8 cm²</td>
<td>9.6 cm²</td>
<td>59 cm²</td>
</tr>
<tr>
<td>Complete Growth Medium</td>
<td>0.5 mL</td>
<td>1.0 mL</td>
<td>2.5 mL</td>
<td>15.5 mL</td>
</tr>
<tr>
<td>Opti-MEM I Reduced Serum Medium</td>
<td>50 µL</td>
<td>100 µL</td>
<td>250 µL</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>DNA (1 µg/µL stock)</td>
<td>0.5 µg</td>
<td>1.0 µg</td>
<td>2.5 µg</td>
<td>15 µg</td>
</tr>
<tr>
<td>GeneXPlus Reagent</td>
<td>1 µL</td>
<td>2 µL</td>
<td>5 µL</td>
<td>30 µL</td>
</tr>
</tbody>
</table>

Notes:
1. EF1α promoter is an optimal promoter for driving expression of gene of interest or GFP in SH-SY5Y cells.
2. Always include a control condition consisting of an empty vector plasmid or a plasmid expressing GFP.

Transfection efficiency of GeneXPlus Reagent on SH-SY5Y human neuroblastoma cells (×10). Cells were transfected with EF1α-eGFP empty vector at 0.5 µg DNA with 1.0 µL of reagent (1:2) in Opti-MEM I Reduced Serum Medium per well of a 24 well plate.