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Package Contents and Storage Conditions

The following components are included:

- One tube (1.0 mL) TurboFectin 8.0 Transfection Reagent (80% ethanol solution)

The above component is shipped at 0°C, but should be kept at +4°C to -20°C for long-term storage (DO NOT FREEZE). If properly stored, it will have a 12-month shelf life.

NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

Related products

- TrueClones
 - Expression-ready full-length cDNA clones covering 98% of human genome. <http://www.origene.com/cDNA>
- HuSH-29
 - Potent shRNA constructs for expression knockdown, covering 100% of human and mouse genomes. <http://www.origene.com/rna/>
- GFC-Transfection Arrays
 - Hundreds or thousands of unique cDNA clones arrayed individually in multi-well plates for high-throughput functional screening. <http://www.origene.com/cdna/gfc-array/>
- Magnetofection reagents
 - Novel transfection reagents that exploit magnetic fields to enhance DNA delivery. <http://www.origene.com/cdna/magnetofection.msp>
 - PolyMag Cat# TT100029, TT100030, TT100031
 - CombiMag Cat# TT100039, TT100040, TT100041
 - SilenceMag Cat# TT100032, TT100033, TT100034, TT100035
 - ViroMag Cat# TT100036, TT100037, TT100038

For technical assistance, contact OriGene at 1-888-267-4436 (301-340-3188 outside the US) or write to us at techsupport@origene.com.

Introduction

TurboFectin 8.0 is a new generation of transfection reagent optimized for nucleic acid delivery into eukaryotic cells. Its proprietary formulation of lipid/histone blend is supplied in 80% alcohol and should be stored at +4°C or –20°C.

TurboFectin 8.0 has been tested and shown to be very effective in the delivery of TrueClones (cDNA clones for gene overexpression) and HuSH-29 constructs (shRNA plasmids to knock down gene expression). TurboFectin 8.0 is also recommended as the reverse transfection reagent in OriGene's novel GFC-Transfection Array application (see reverse transfection in the Application Protocols section.)

The major advantages of TurboFectin 8.0 include:

- **High efficiency:** Comparable with the leading brand FuGENE6
- **Low toxicity:** Comparable with the leading brand FuGENE6
- **Simple application:** Works well in media containing antibiotic and antimycotic agents. Suitable for serum-containing media; no requirement for media changes.
- **Wide spectrum:** Over 100 cell lines and primary cell types have been successfully transfected with TurboFectin 8.0 (See [Turbofectin 8.0 Cells](#) for list)
- **Affordability:** One mL of TurboFectin can typically be used for 500 transfections of 6-well plate cultures. The cost is less than \$0.50 per transfection.

Production and Quality Assurance:

TurboFectin 8.0 is lot tested against standard cell lines to assure consistency and efficacy.

Experimental Procedures

Transfection Optimization

Although the transfection protocols below have been proven to result in highly efficient transfection, it is encouraged to carefully optimize the reaction conditions for each individual cell type. The following variables should be considered:

- A. Cell density (% confluence at transfection): The recommended confluence for most cell types at transfection is 50-70%. Determine the optimal cell density for each cell type for maximal efficiency and maintain the density in all experiments for reproducibility.
- B. Media conditions: TurboFectin 8.0 performs better in complete growth medium compared to serum-free medium. No media change is necessary before or after the transfection.
- C. DNA purity and concentration: OriGene recommends highly purified, sterile DNA prepared on ion-exchange columns. It is important to remove the contamination of endotoxin from the DNA prep for maximal transfection efficiency. The optimal DNA concentration for transfection is 1-3 ug per well for a 6-well plate. Refer to Table 1 for more details.
- D. TurboFectin 8.0 to DNA ratio: The standard ratio is 3 uL of TurboFectin to 1 ug of DNA. It is recommended to titrate the ratio by adjusting the TurboFectin from 2-8 uL per 1 ug DNA. Refer to Table 1 for more details.

Table 1. Recommended starting transfection conditions for TurboFectin 8.0

Culture plate	10cm Dish	6-well	12-well	24-well	48-well	96-well
surface area (cm ²)	59	9.6	3.8	1.9	1.0	0.35
volume of complex (uL)	600	100	50	20	10	5
TurboFectin 8.0 (uL)	18	3	1.5	0.6	0.3	0.15
DNA volume (uL; 1ug/uL stock)	6	1	0.5	0.2	0.1	0.05

Application Protocols

Protocol for transient transfection (adherent cells)

A sample protocol is listed here for experiments performed in 6-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

1. Cell Plating
 - a) On the day before transfection, plate cells at a density of $1-3 \times 10^5$ cells in complete growth medium per well of a 6-well plate to obtain 50-70% confluence on the following day.
 - b) Incubate overnight.

2. Complex formation (perform this step immediately before transfection)
 - a) In a sterile plastic tube, add 100 uL of **serum free** medium.
 - b) Add the appropriate amount of TurboFectin 8.0 (2-6 uL per 1 ug DNA) into tube. Mix completely by gentle pipetting.
 - c) Incubate at room temperature for 5 minutes.
 - d) Add plasmid DNA (1-3 ug per well) to the TurboFectin-containing media prepared above. Mix by gentle pipetting.
 - e) Incubate at room temperature for 15-30 min.

3. Transfection in complete culture medium
 - a) If necessary, remove spent medium from the cells prepared in Step 1 and replace with 2 mL of fresh complete medium per well.
 - b) Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the dish to distribute the complex evenly.
 - c) Incubate for 24-48 hours.

Note: With TurboFectin, no medium change is necessary. If you wish to remove the complex, remove the medium 4-24 hours post-transfection and replace with fresh complete medium.

Protocol for transient transfection (suspension cells)

A sample protocol is listed here for experiments performed in 6-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

1. Cell Plating

- a) On the day before transfection, plate cells at a density of $5-10 \times 10^5$ cells in complete growth medium per well of a 6-well plate for optimal cell density on the following day.
 - b) Incubate overnight.
2. Complex formation (perform this step immediately before transfection)
 - a) In a sterile plastic tube, add 250 μ L of **serum free** medium.
 - b) Add the appropriate amount of TurboFectin 8.0 (2-6 μ L per 1 μ g DNA) into tube. Mix completely by gentle pipetting.
 - c) Incubate at room temperature for 5 minutes.
 - d) Add plasmid DNA (1-3 μ g per well) to the TurboFectin-containing media prepared above. Mix by gentle pipetting.
 - e) Incubate at room temperature for 15-30 min.
 3. Transfection in complete culture medium
 - a) If necessary, spin down the cells prepared in Step 1 and remove spent medium. Resuspend the cells with 2.5 mL of fresh complete medium per well.
 - b) Add the mixture prepared in Step 2 dropwise to the cells. Gently rock the dish to distribute the complex evenly.
 - c) Incubate for 24-48 hours.

Note: With TurboFectin, no medium change is necessary. If you wish to remove the complex, remove the medium 4-24 hours post-transfection and replace with fresh complete medium.

Protocol for stable transfection:

Perform a transfection as described above. Twenty-four hours post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing selective medium. A mock transfection plate should be used in parallel as control.

Protocol for reverse transfection:

In conventional transfection, the cells are in the plate before the DNA/transfection reagent is applied. When the plasmid DNA is applied to the plate before the cells are, such transfection is referred to as **reverse transfection**.

Reverse transfection is usually utilized in high-throughput applications when a large number of individual plasmid DNAs is arrayed onto a matrix, such as a glass slide or a multi-well plate. For example, in OriGene's GFC-Transfection Arrays, hundreds to thousands of individual clones are arrayed into 384-well plates and lyophilized. Cells and reporters are then added to these wells. TurboFectin 8.0 is the preferred reverse transfection reagent for GFC-Transfection Array.

Table 2. The reference points for reverse transfection protocols using multi-well plates

Contents (per well)	96-well	384-well
plasmid DNA (ng)	100-125	30-60
TurboFectin 8.0 (uL)	0.3-0.38	0.09-0.18
complex volume (uL)	10-20	10
number of cells	10,000-20,000	5,000-7,500
final volume (uL)	up to 200	up to 60

For different cell lines, optimization is necessary to get the best TurboFectin (uL): DNA (ug) ratio. Three ratios are recommended for initial testing: 3:2, 3:1 and 6:1.

The protocol below describes the application using OriGene's standard GFC-Transfection Array in 384-well plates (60 ng DNA per well) and using a TurboFectin:DNA ratio (uL:ug) of 3:1. For 96-well plates, simply use the recommended volumes from Table 2 above.

1. Rehydration of the dried DNA in the plate.
Equilibrate the sealed plates and serum-free medium to room temperature. If a reporter plasmid is needed, add the desired volume of the reporter into 4 mL of the serum free medium. Dispense 10 uL of the reporter/medium into each well using a multi-channel pipettor. Shake the plate on a microplate mixer for 10 minutes and collect the solution by brief centrifugation.
2. Preparing the diluted TurboFectin 8.0.
For each 384-well plate, prepare 4 mL of serum-free medium in a sterile tube. Add 80 uL of TurboFectin 8.0 into the medium and mix thoroughly by pipetting up and down.
3. Dispense 10 uL of the transfection reagent into each of the wells using a multi-channel pipettor. Shake on a microplate mixer for 30 seconds and then centrifuge briefly to collect the solution.
4. Incubate at room temperature for 20-45 minutes for DNA/TurboFectin complex formation.
5. During the incubation time, prepare the cells to be transfected.
 - The cells should be at confluency lower than 75% (for HEK293 cells).
 - Remove the spent media. Rinse once with 5 mL sterile PBS.
 - Add 1-5 mL (depending on the size of flask used) of pre-warmed Trypsin/EDTA medium to the cells. Incubate at 37°C for 5min. If trypsinization will harm your cells or assay, 10 mM EDTA in PBS (without Ca²⁺ or Mg²⁺) can be used.
 - Remove the trypsinized cells to a sterile 15 mL conical centrifuge tube and add pre-warmed complete DMEM media. Mix the cells well (or vortex) and centrifuge at 1600 RPM for 5 minutes.
 - During the entire procedure, make sure that the cells are well separated from each other (single cells). Vortex the cell if possible or necessary.
 - Aspirate the liquid leaving a small amount of media on the pellet for resuspension of the cells. Resuspend the cells by flicking the tube and add 10 mL of pre-warmed **complete** DMEM media. Mix the cells well (or vortex) and centrifuge at 1600 RPM for 5 minutes.

- Resuspend cells in 5 mL of pre-warmed **complete** DMEM media.
 - To determine cell concentration, add 10 uL to a hemocytometer and count cells in 2 of the 1mm² squares. The cell concentration is $(X1+X2)/2 \times 10^4$ cells/mL.
 - Adjust the cell density to about 250,000 cells /mL, or to the optimized concentration of cells, with complete DMEM medium.
6. At the end of the incubation, aliquot 30 uL of cell suspension into each well with an electronic multi-channel pipettor. Let cells settle by gravity. Do not vortex, mix or spin down the cells (unless required by the assay).
 7. Cover the plate and incubate at 37°C in a CO₂ incubator for 48 hours.

Trouble-shooting Guide

Low cell viability or low cell growth rate

Possible cause: Complexes were added to the cells in **serum-free** media

Solution: Form complexes in serum-free media and add to cells in complete growth media.

Possible cause: Cell density was not optimal at the time of transfection

Solution: Optimize transfection by testing various cell confluencies in small-scale experiments. Maintain a consistent density in future experiments to ensure reproducibility.

Possible cause: TurboFectin/DNA complex was not mixed thoroughly with the cells.

Solution: Rock the dish back and forth and from side to side to ensure even distribution of the complexes onto the cells. Do not swirl or rotate the dish as such motion can result uneven distribution.

Possible cause: TurboFectin concentration is too high

Solution: Reduce the amount of TurboFectin, and optimize the ratio of TurboFectin:DNA to minimize toxicity.

Low transfection efficiency

Possible cause: TurboFectin/DNA ratio is not optimized

Solution: Titrate the reagent from 2-8 uL per 1 ug DNA. Select the ratio with best transfection and the lowest toxicity for future experiments.

Possible cause: Complexes were added to the cells in serum-free media

Solution: Form complexes in serum-free media and add to cells in complete growth media.

Possible cause: Complexes were formed in serum containing media.

Solution: Form complexes in serum-free media and add to cells in complete growth media.

Possible cause: Presence of polyanions, such as dextran sulphate or heparin, in the media.

Solution: Use a growth medium that does not contain these polyanions.

Possible cause: Insufficient incubation time during complex formation

Solution: Incubate the TurboFectin/DNA/serum free medium solution for 15-30 minutes prior to adding it to the target cells. Individual cell types may require optimizing this incubation time for maximal transfection efficiency.

Possible cause: Poor quality DNA

Solution: The DNA used for transfection should be highly purified, sterile, endotoxin-free material for optimal transfection efficiency.

Frequently Asked Questions

Q: What kind of reagent is TurboFectin?

A: TurboFectin is a proprietary mixture of a broad-spectrum protein/polyamine with histones and lipids.

Q: Can TurboFectin be used to transfect primary cells?

A: Yes, a number of primary cells have been successfully transfected with TurboFectin. See the complete list of successfully transfected cells on our website at [TurboFectin 8.0 cell lines.xls](#).

Q: My cells are grown in serum-free medium. Can I still use TurboFectin to transfect them?

A: Yes. While TurboFectin shows the best transfection efficiency in medium containing serum, it is also effective at transfecting cells grown in medium that does not contain serum.

Q: Will the use of antibiotics in my growth medium interfere with transfection efficiency?

A: While TurboFectin has been shown to be effective in the presence of antibiotics, if you have low transfection efficiency you may wish to test the protocol again in the absence of antibiotics. Certain cationic antibiotics (such as kanamycin) may decrease the efficiency of TurboFectin transfection.

Q: Why has my TurboFectin developed a precipitate during storage?

A: Storage at -20°C may cause some precipitation in the TurboFectin, but this is completely normal and reversible. Simply warm the reagent to room temperature and gently vortex before use. You may store TurboFectin at +4°C to avoid the development of precipitates.

Q: How long do I need to leave the TurboFectin – DNA solution on my cells before assaying them?

A: We recommend a minimum of 24 hours of incubation prior to assay. Depending on your assay, a longer time (48-72 hours) may be required to produce the necessary effect, but the transfection solution can be removed from the cells after 24 hours if necessary. However, a medium change is not necessary, so the transfection solution can remain on the cells for 48-72 hours.