HyFectin $LD_{1.0}$ DNA Transfection Reagent

Catalog Number: Che_0514

Feature

1. Simple transfection protocol- ready to use, just mix DNA and vortex.
2. Superior transfection efficiency within FCS medium.
3. Effective for both adherent and suspension cells.
4. Minimal cytotoxicity, excellent choice for human ES cells transfection.
5. No medium change after transfection.
6. Highly reproducible results.
7. Products stable at both 4°C and - 20°C.

Kit Components

1. HyFectin $LD_{1.0}$ solution: 1 ml, supplied as a sterile filtered solution
2. Dilution Buffer: 30 ml
3. Transfection-ready Control DNA: 1 µg CMV_eGFP DNA in 10 µl TE buffer.

Description

HyFectin $LD_{1.0}$ is a new generation of polymer-based plasmid DNA transfection reagent optimized for DNAs delivery into mammalian cells to provide following advantages:

- High transfection efficiency in many cell types and formats. Comparable with the leading brand Lipofectamine 2000 / FuGene 6.
- Lower toxicity than the leading brands Lipofectamine 2000 / FuGene 6.
- There is no need to remove DNA-HyFectin complexes or change medium after transfection.
- The transfection complexes can be added directly to cells in culture medium, in the presence or absence of serum.
- Affordability: One ml of HyFectin $LD_{1.0}$ can typically be used for 330 transfections when using 1µg plasmid DNA per well of a 6-well culture plate.

The ratio of HyFectin $LD_{1.0}$ to plasmid DNA is fixed at 3 µl HyFectin $LD_{1.0}$ for 1µg plasmid DNA in 30 – 50 µl of dilution buffer. HyFectin $LD_{1.0}$ is formulated in a ready-to use format. All users need to do is to add DNA and mix. After 15 minutes room temperature incubation, the transfection mix can be added to cells.

Protocol for transfection of adherent cells

1. Add 1µg DNA into 50 µl dilution buffer, then add 3 µl of HyFectin $LD_{1.0}$ and Mix well.
2. Incubate the mix at room temperature for 15 minutes.
3. Add the mix to culture cells.

Table Calculation of maximal plasmid DNA loading per transfection

Culture Vessel	**Surface Area** per well	**Medium** per well	**DNA** per well	**HyFectin LD$_{1.0}$** Per well
96 well	0.3 cm^2	0.2 ml	0.25 µg	0.75 µl
24 well	2.0 cm^2	1 ml	1 µg	3 µl
12 well	4.0 cm^2	2 ml	2 µg	6 µl
6 well	9.6 cm^2	4 ml	4 µg	12 µl
10 cm	60 cm^2	15 ml	20 µg	60 µl

The quantity of highly purified plasmid DNA is the most important parameter in transfection. Not using sufficient amount of DNA will result in reduced transfection efficiency, at the same time, using too much DNA will generate toxicity. The ratio of HyFectin LD$_{1.0}$ is optimally fixed at 1:3, and should not be changed. Change of medium in 12 to 24 hours post transfection may improve cell growth condition, and is recommended. With HyFectin LD$_{1.0}$, change of medium after transfection is not absolutely required.

Protocol for transfection of suspension cells

HyFectin LD$_{1.0}$ has proven to be an effective reagent to transfect suspension cells. The ideal cell density at time of transfection is 1 X 10^6 / ml. Therefore, the day before transfection, suspension cells, such as CHO and 293T cells, are seeded at 0.5 X 10^6 / ml in culture medium (DMEM/F12 medium gives the best result) without serum. The amount of DNA used in the transfection should be 3µg plasmid DNA per ml of cells, which yields optimal cell growth and target protein expression. Transfection mix can be prepared exactly as described in the previous section. The transfection mix is then added to the suspension cells. Media replacement is recommended in 12 to 24 hours post transfection, and data has shown that media replacement can increase cell growth rate after transfection for suspension cells. For protein or antibody production, cell media can be harvested 4-7 days post transfection.

Cell lines and plasmid DNAs are all different from each other. The protocol and recommendations described here are optimized for CHO cell line. Tests should be run to optimize the transfection conditions and the quantity of expression plasmids for different cell lines.

Protocol for transfection of human ES cells

Most transfection reagents require certain cell seeding density (20-60%) to obtain its maximal transfection efficiency. To prepare single ES (or iPS) cell as seeding is a critical step in the DNA transfection protocol to obtain a stable ES cell line.

1. Cultivate human ES or iPS cell in recombinant human vitronectin coated plate (6 well plate) with xeno-free Nutristem medium.

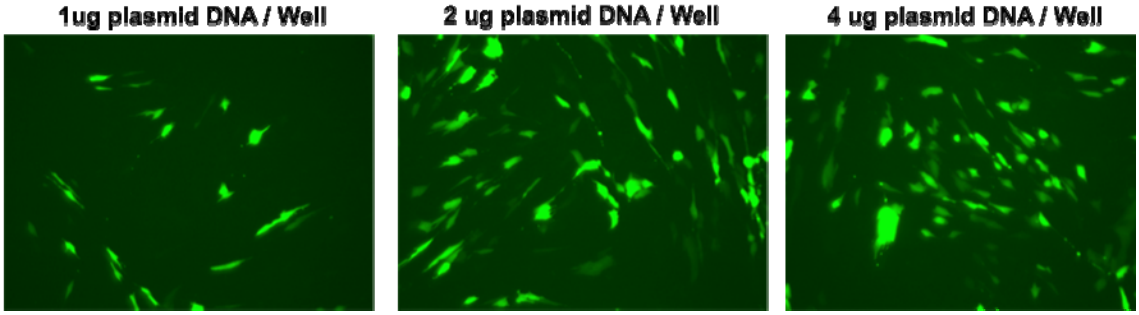
2. Wash cell once with pre-warmed PBS solution, trypsinize cell sample (1 ml trypsin solution per well) for 3 minutes at room temperature. Single cells could be obtained by gently pipetting cell clumps with a P100 pipet.
3. Add 3 – 6 ml NutriStem medium containing 10% FBS (or 10% human serum) to neutralize trypsin before counting cell number.
4. Spin down cells at 1000G for 3 minutes, re-suspend cells at 0.25 x 10^6/ml in NutriStem medium (which supplemented with Stemgent's hESC cloning & recovery supplement [cat# 01-0014] at 1:1000 dilution). Seed 2 ml cell suspension per Vitronectin coated well, and incubate at 37°C overnight.
5. Add 1 µg endo-free plasmid DNA with 3 µl HyFectin $LD_{1.0}$ reagent in 50 µl dilution buffer per well. 25 – 50% transfection efficiency was routinely obtained with pCMV-eGFP control plasmid.

Additional materials required for human ES cell transfection

- Recombinant Human Vitronectin: Cat# HRP-0299, LD Biopharma, Inc.
- hES cell Cloning & Recovery Supplement: Cat# 01-0014-500, Stemgent, Inc.
- NutriStem Medium: Cat# 01-0005, Stemgent, Inc.

Storage

Store at – 20 °C or 4°C. Stable for 12 months from the date of shipment.



pCMV-eGFP plasmid was purified using Qiagen mini-preparation kit, and transfected into 6 well plate with various amount of plasmid DNA for human primary fibroblast cells in 10% FCS / DMEM media. Data was collected on following day after transfection.