

Note: Store dehydrated Glycofect Transfection Reagent at -20°C. Do not rehydrate until ready to use.

Product Features

Glycofect™ transfection reagent is a biodegradable nucleic acid carrier consisting of a blend of carbohydrate-based cationic polymers, that combines with DNA to form nanoparticles. Glycofect™ is not of animal origin and is specifically designed for low toxicity transfection of DNA in eukaryotic cells.

- Specifically optimized for DNA delivery due to efficient nuclear delivery.
- Low cytotoxicity and higher protein expression compared with polyamine and lipid-based transfection reagents.
- Formation of complexes between DNA and Glycofect transfection reagent is simple. Complexes biodegrade after endocytosis and promote high transgene expression without cell membrane damage.

Transfection Information

- This protocol is written for **suspension cells** in 24-well plate experiments, but can be scaled to other formats (e. g. 6-well, see page 3)
- The pre-aliquot dose is generally sufficient for the most optimized balance between protein expression and toxicity. ***It is highly recommended that a dose-optimization procedure is performed:*** Dilutions (1.5x, 2x, or 2.5x) of this solution can be prepared with ultrapure water (prior to polyplex formation) and used (following the same volume recommendations listed below) to optimize gene expression vs. toxicity.
- Unlike lipid-based transfection reagents, experiments can be carried out in antibiotic containing media
- Glycofect is most effective when transfection is initiated for at least 4 h in serum free media
- Because of this product's biodegradable nature, *excess dissolved transfection reagent should be discarded and not saved for later use.*
- Additional details and other specific information can be found at our website: www.Techulon.com

Transfection Protocol

Use this procedure for transfection of DNA into suspension cells using the 24-well plate format. Each vial of Glycofect transfection reagent contains enough material for an entire plate. Discard any excess reagent after the experiment.

1. Cells should be plated 24 hours prior to transfection at a typical seeding density of $1-5 \times 10^5$ cells per well (media volume = 500µl) depending on cell size. Thirty

minutes preceding transfection, Glycofect transfection reagent should be dissolved in 1,250 µl of ultrapure DNase/RNase free water.

2. DNA solutions should also be diluted for optimal DNA complex formation. Dilute all DNA solutions to 0.05 – 0.20 µg/µl in ultrapure DNase/RNase free water to facilitate optimal particle formation. Testing multiple concentrations in this range is recommended.
3. **For each well:** Add 50 µl of prepared Glycofect solution to 50 µl of DNA solution ([pDNA] = 0.05 – 0.20 µg/µl; total DNA amount per well = 2.5 - 10 µg) and mix thoroughly with pipette flushing in an eppendorf tube or 96-well plate. We recommend testing multiple DNA concentrations and Glycofect concentrations to determine optimized gene expression conditions. Glycofect /DNA complexes should incubate at room temperature for 30 minutes making a formulation that is stable for 2 hours. Once complexes have been formed proceed to step 5.
4. For best transfection efficiency cells should be suspended in RPMI or ProCHO medium without serum for the first 4 h of transfection.
5. Add 100 µl of Glycofect/DNA solution directly to the media in each well, gently swirl the plate, and return cells to incubator.
6. After 4 hours, it is optional to add 500 µl more media (serum containing if appropriate) and return cells to incubator and incubate according to cell line instructions.
7. Protein production can be assayed at 24 h, but maximum protein production occurs between 2 – 7 days. This should be optimized with a reporter gene.

Variables to Consider When Scaling Transfections

- When forming the DNA-Glycofect complex:
 - Always perform optimizations utilizing different concentrations of DNA and Glycofect
 - [DNA] = 0.05 – 0.20 µg/µl
 - Use 100 µl of Glycofect solution and 100 µl of DNA solution (200 µl total volume) per every 1 ml of cells.
- Larger formats may require slower spinning to reduce foam formation, etc.
- Positive and negative controls should be implemented in all pilot studies.

Technical Support and Information

Telephone support is available from 9 AM to 5 PM weekdays U.S. Eastern Time:

540-443-9254

Email your problem or question to: Glycofect@techulon.com