

Technical Manual NOVAFACT

NOVAFACT O15/O25/G214 PRODUCT INFORMATION SHEETS

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1 Product description

NOVAFACT O transfection reagent kit contains proprietary linear, cationic and water-soluble chitosan based oligomers and polymers that easily form polyplexes with DNA through electrostatic interactions [1,2]. In contrast to many other polycations, the polyplexes formed by chitosan with pDNA exhibit low cytotoxicity and have been used for delivery of genes to mucosal tissues *in vivo* [3]. The ability of chitosan polyplexes to transfect cells has been documented in different cell cultures *in vitro* and animal models *in vivo* [3,4].

The NOVAFACT O kit contains 3 vials of 10 mg of highly purified, sterile-filtered and lyophilized material (see also separate specification sheet):

NOVAFACT O 15	Linear oligomers with DP _n (number average degree of polymerization) of 15-24, degree of deacetylation ≥97%.
NOVAFACT O 25	Linear oligomers with DP _n of 25-40, degree of deacetylation ≥97%.
NOVAFACT G 214	Polymeric water-soluble chitosan glutamate salt, degree of deacetylation ≥90%.

Add sterile solvent to the vials to obtain the appropriate concentration of NOVAFACT (see "Suggested protocol for use" below).

NOVAFACT-pDNA complexes (polyplexes) form spontaneously upon addition of pDNA under mixing, and formulations of these polyplexes may be directly added to cells (see "Suggested protocol for use" below).

NOVAFACT products are released as Research Products (not intended for human use). The NOVAFACT O transfection reagent kit is intended for exploring and first optimization of transfection protocols for different cell lines and different nucleic acids. Each vial of 10 mg contains sufficient material for 1000-5000 transfections in 96-well plates (see "Suggested protocol for use").

NOVAFACT O transfection reagent kit should be stored at 2-8°C. Stock solutions may be stored at -20°C for minimum one year.

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2 Sample data

NOVAFACT-pDNA polyplexes may have different shapes and sizes depending on experimental conditions, soluble and globular structures being favored by increased protonation of the cation [2].

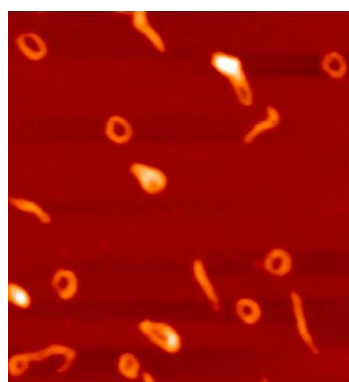


Figure 1: Atomic Force Microscopy shows the formation of NOVAFECT-pDNA polyplexes of about 50 nm in size.

Agarose gel retardation assay may be used to monitor polyplex formation and polyplex stability. While naked pDNA will migrate in the gel, the stable NOVAFECT:pDNA complexes will be retained in the wells. Typically, stable polyplexes are formed at charge ratios of NOVAFECT:pDNA in the range 10:1 to 60:1 (Figure 2), depending on the chain length (DP_n) of the oligomer and the pH of the electrophoresis buffer.

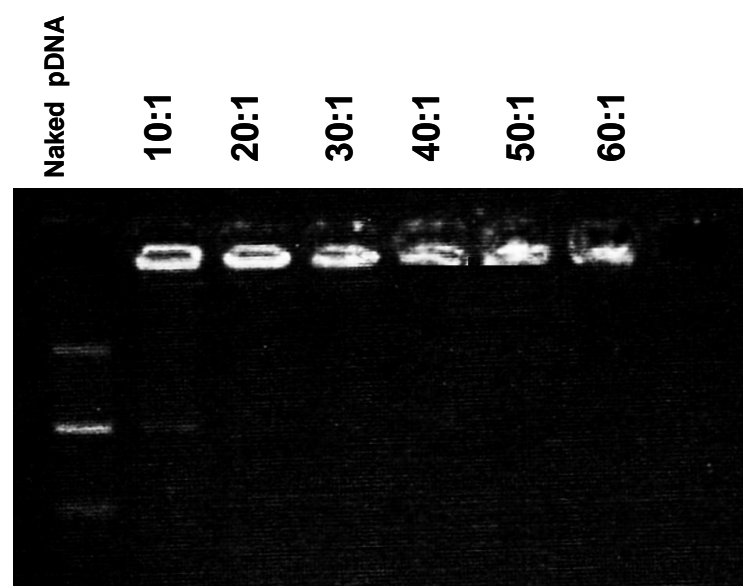


Figure 2: Gel agarose assay showing NOVAFECT:pDNA complexes at charge ratio 10:1 to 60:1.

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Transfection *in vitro*:

The following cell lines have been shown to be transfected with oligomeric (NOVAFACT O15/O25) and polymeric products (NOVAFACT G214), with no acute toxicity observed [3,4]:

- HEK 293, human embryonic kidney
- Calu-3, airway epithelial
- HeLa, cervix epithelial

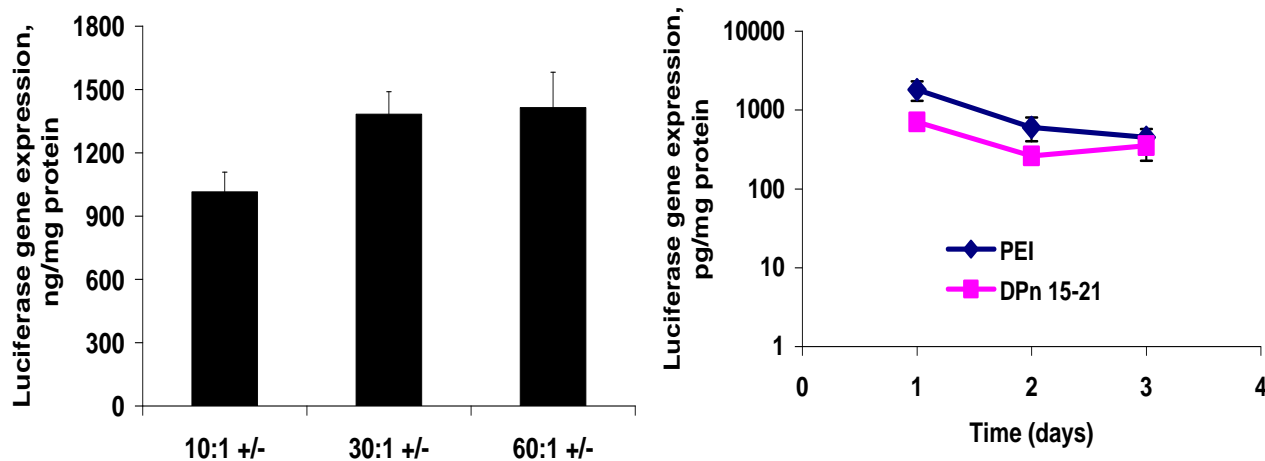


Figure 3: Luciferase gene expression in (left) HEK293 cells 48 h following transfection with NOVAFACT O 25, and (right) in the mouse lung *in vivo* following transfection with highly fractionated low molar mass chitosan oligomers (for details, see reference [4]).

Transfection *in vivo*:

NOVAFACT O15/O25 are particularly suitable for transfection of mucosal tissues.

Efficient *in vivo* luciferase gene expression in mouse lungs has been demonstrated following intratracheal administration, with no acute toxicity observed [3,4] neither for oligomeric products (NOVAFACT O15/O25), nor for polymeric products (NOVAFACT G214).

3 Protocol for use

Given below is an example protocol for transfecting adherent HEK293 cells in 96-well plates, using NOVAFACT O15/O25.

Perform all steps of protocol in a laminar flow cell culture hood using sterile techniques.

Preparation of NOVAFACT: pDNA polyplexes

1. Prepare stock solution of 2 mg/ml of NOVAFACT O15/O25 in sterile ultrapure water.
2. Prepare stock solution of 0.5 mg/ml of plasmid (pDNA).

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3. Add pDNA stock solution to ultrapure water, and add the NOVAFACT solution to the pDNA/water mixture under vortex mixing (1200 rpm) for at least 10 seconds. Guidance for the composition of the formulations is shown in Table 1.

Table 1: Suggested composition of polyplexes. All calculations are shown for quadruplicate samples, pDNA concentration of 13.2 µg/ml, corresponding 0,33 µg pDNA/well. (If concentration of pDNA higher than 13.2 µg/mL is requested, we recommend mixing NOVAFACT reagent and pDNA in approx. 1:1 v/v ratio to avoid aggregation).

	Formulation charge ratio (plasmid: NOVAFACT)*					
	1:10	1:20	1:30	1:40	1:50	1:60
Plasmid (0.5 mg/ml)	2,64 µl	2,64 µl	2,64 µl	2,64 µl	2,64 µl	2,64 µl
NOVAFACT (2 mg/ml)	4,42 µl	8.84 µl	13.3 µl	17.7 µl	22.1 µl	26.5 µl
Ultrapure water	92.94 µl	88.52 µl	84.06 µl	79.66 µl	75.26 µl	70.86 µl
Total volume	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl

*The ideal formulation charge ratio depends on the NOVAFACT type used and should be always determined in each application. For NOVAFACT O15 and O25, we recommend 1:30-1:60 and 1:10-1:30, respectively. For NOVAFACT G214, charge ratios < 1:5 are usually required.

4. Incubate the polyplexes for 30 min.
5. After incubation, the polyplexes are diluted to 200 µL with transfection medium as described in 2 below.

Transfection in 96-well plates

The following is a suggested protocol for transfection of HEK293 cells in 96-well plates. The optimal seeding density will vary with growth characteristics of the cell line.

1. Seed cells (approx. 30 000 cells/well) 24 h before transfection to reach 70-90% confluency at the time of transfection.
2. Prepare transfection formulations as 1:1 mixtures of NOVAFACT/pDNA polyplexes and hypertonic (580 mM) serum-free medium (OptiMEM I, Gibco containing 20 mM Hepes and 270 mM mannitol), so that the final formulation is isotonic and contains 6.6 µg/ml pDNA. **It is crucial that the pH of the hypertonic OptiMEM may not exceed pH 7.4.**
3. Wash the cells carefully with 200 µL preheated OptiMEM.
4. Add 50µL of the transfection formulation containing a dose of 0,33 µg pDNA to each well. Each formulation should be used at least in triplicate for statistical analysis.
5. Incubate at 37°C in 5% CO₂, for 5 hours.
6. Replace the transfection mixture with 200 µL of fresh culture medium.
7. Incubate with regular medium changes at 37°C in 5% CO₂ for 48-96 hrs prior to protein analysis.

Product Information Bulletin



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4 Order information

NOVAFACT O test kit can be ordered directly on the web at www.novamatrix.biz using credit card payment, or by fax to +47 67815510 attentioned to Kristin Hefte, or by e-mail to kristin.hefte@fmc.com.

NOVAFACT products are sold under a Supply Agreement with IP protection. Patents are pending in all major markets. License opportunities are available.

References:

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Technical Service

The information contained in this bulletin is intended to be general in nature. Techniques and data pertaining to specific uses for NovaMatrix/FMC products and new developments will be published periodically in the form of supplemental application bulletins. Our technical staff is ready to offer assistance in the use of NovaMatrix/FMC products.

Regulatory Status

NOVAFACT products are chitosan-based, they are classified as research products, and are not intended for human use.

PROTASAN™ chitosan salts satisfy the requirements for use in tissue-engineered medical products as set forth in ASTM F2064, ASTM F2103 and ASTM F2347.

PROTASAN™ chitosan products are manufactured in compliance with cGMP and described in separate DMF's submitted to the US FDA.

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