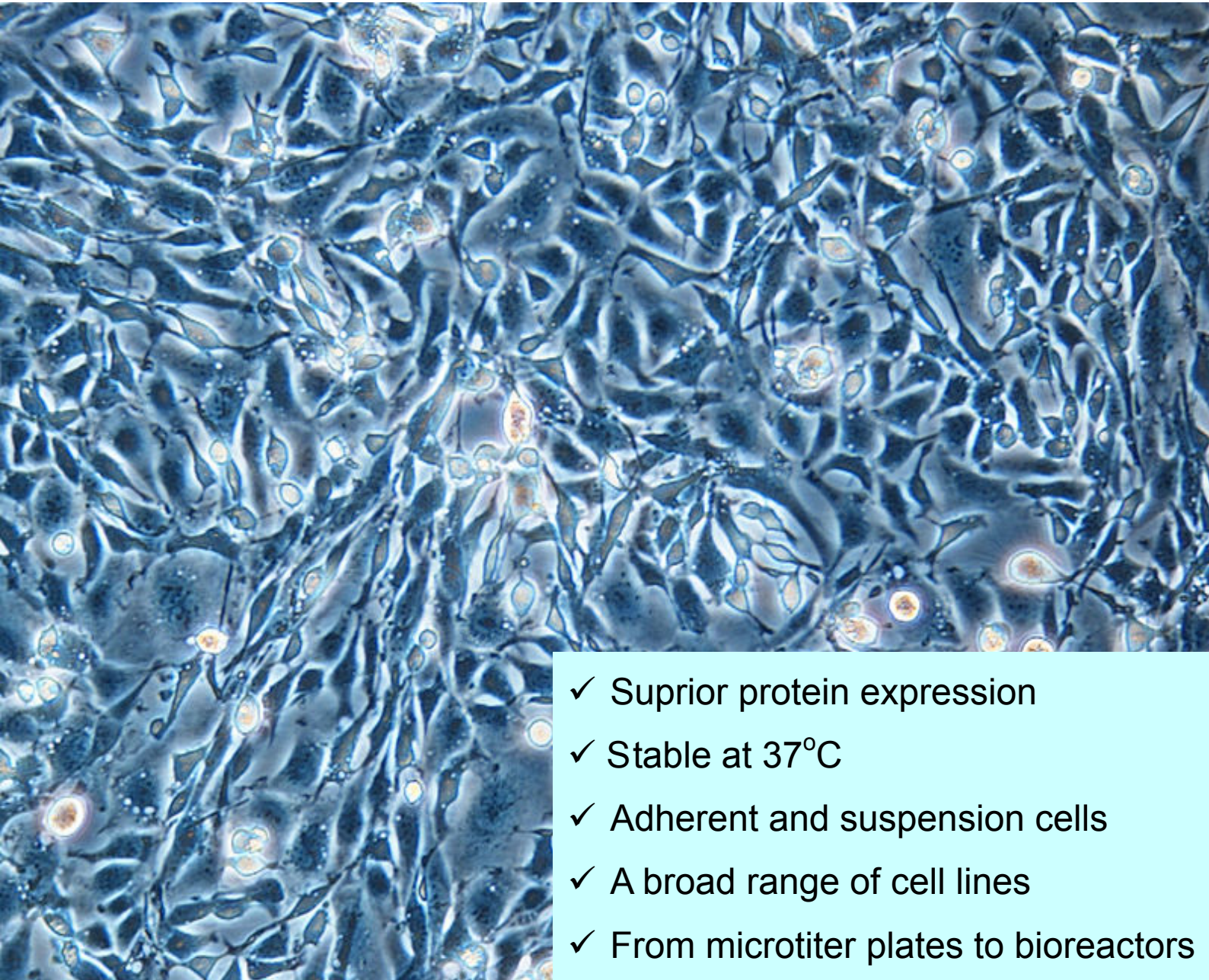




Sino Biological Inc.
Biological Solution Specialist

Sinofection

Transfection Reagent



- ✓ Superior protein expression
- ✓ Stable at 37°C
- ✓ Adherent and suspension cells
- ✓ A broad range of cell lines
- ✓ From microtiter plates to bioreactors

Introduction to Sinofection

For DNA transfection of broad range of cell lines

Sinofection is a superior cationic polymer-based transfection reagent. It has been successfully applied to transfection at various scales over a broad range of cell lines. Compared with the leading transfection reagents on the market, Sinofection yields higher protein expression level and exhibits lower cytotoxicity. It is an ideal choice for recombinant protein production for both transient and stable transfection. Moreover, the stability study of Sinofection showed satisfactory stability at -20°C ~37°C for at least 6 months. Its extensive compatibility with serum, cell type, scale, and storage temperature offers you ease and assurance for research and production.

Features

- **Superior protein expression**
- **Excellent transfection of both adherent and suspension cells for a broad range of cell lines**
- **Remarkable application of DNA transfection (transient/stable) at scales from microtiter plates to bioreactors**
- **Striking stability at 37°C**
- **Easy and fast procedure**
- **Low cytotoxicity**

Superior protein expression

Transfection with Sinofection has been proved to provide higher levels of protein expression in several cell lines than leading competitor's products. For large-scale production of recombinant proteins, Sinofection is the best choice to achieve the optimum yield. In addition, Sinofection also exhibits high transfection efficiency to ensure successful transfections.

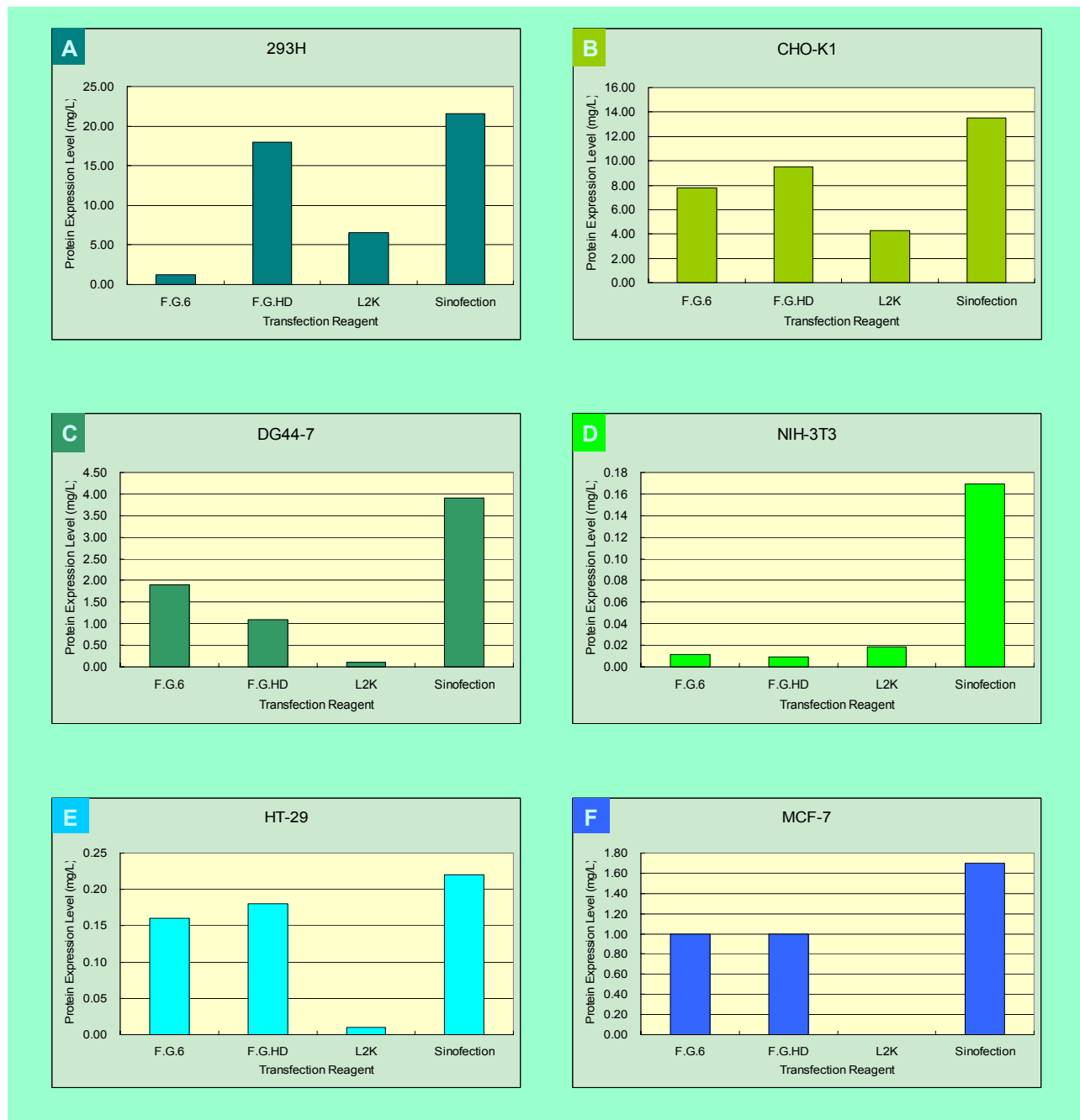


Fig. 1 Protein expression levels by transient transfection using Sinofection and other competitors were compared in 9 cell lines, 6 of which showed superior results transfected by Sinofection (A~F). Protein expression level was determined by Elisa assay. The transfection by competitor transfection reagents followed the manufacturers' protocols.

Excellent transfection of both adherent and suspension cells for a broad range of cell lines

Table 1. Cell lines successfully transfected with Sinofection

Cell line	Cell type
293E	Human embryonic kidney
293H	Human embryonic kidney
CHO-K1	Chinese hamster ovary
DG44	Chinese hamster ovary
HeLa	Human cervical carcinoma
NIH-3T3	Mouse embryo fibroblast
HT-29	human colon adenocarcinoma
Vero	Monkey kidney
MCF-7	human breast adenocarcinoma
A549	Human lung carcinoma
SF9	Ovary, <i>Spodoptera frugiperda</i>

Remarkable application of DNA transfection (transient/stable) at scales from microtiter plates to bioreactors

Hundreds of recombinant antibodies and proteins have been successfully expressed by transient or stable transfection with Sinofection at various scales, from 0.1ml in a 96-well plate to 50L in a bioreactor. Utilizing Sinofection for all scales of transfection application will save the cost of research and production to a great extent.

Striking stability at 37°C

Long-term stability test showed Sinofection remained its activity at 37°C for at least 4 months. Storage at -20°C~RT can maintain stable for at least 6 months. Exceptional stability resulted from rigorous screening and reliable investigation for a long time. Thus the stability of Sinofection can outperform any other transfection reagent.

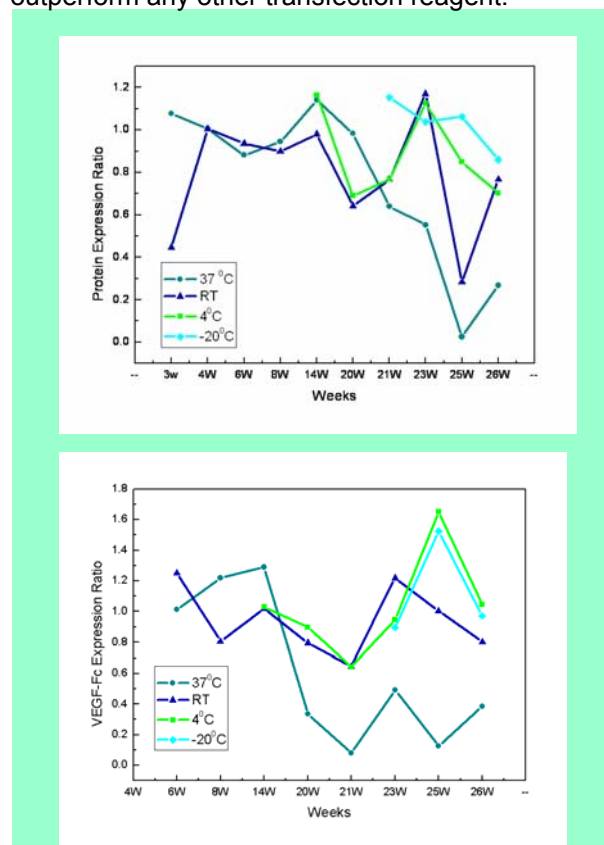


Fig. 2. Stability test for Sinofection at 4 temperatures during 26 weeks. One recombinant antibody and recombinant VEGF-Fc were tested for expression level by transient transfection with Sinofection stored under different conditions. Expression level was assayed by Elisa.

Table 2. Scaling up or down transfections with Sinofection. **Note:** the actual conditions should be optimized by experiments.

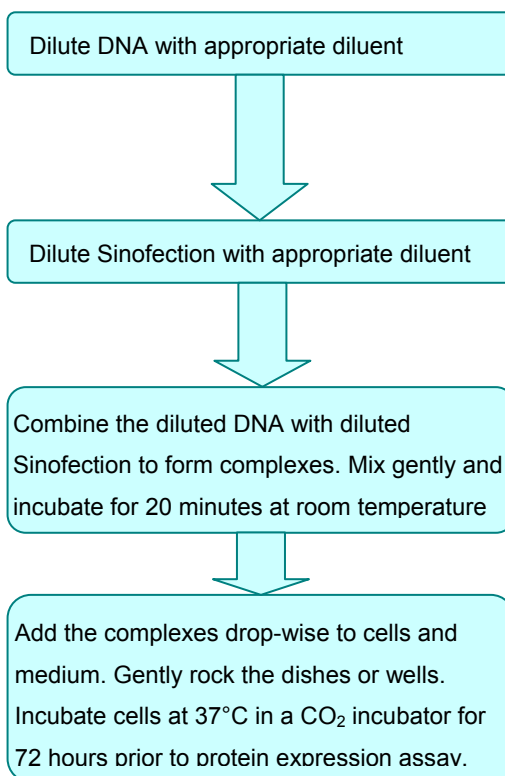
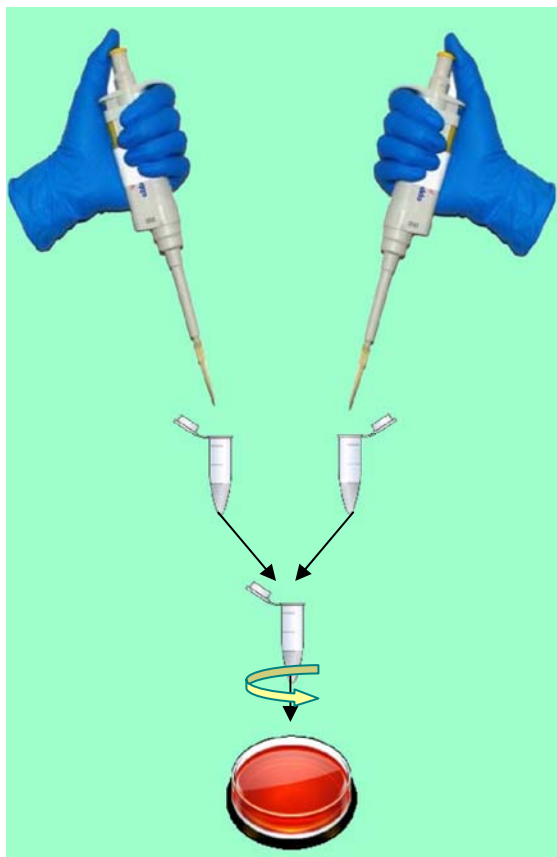
Culture vessel	Vol. of medium(ml)	Growth area (cm ² /well)	DNA (μg/well)	Volume of Sinofection (μl/well)
96-well plate	0.1	0.3	0.05~0.2	0.25~0.75
48-well plate	0.2	0.7	0.1~0.4	0.5~1.5
24-well plate	0.5	2	0.2~0.8	1~3
12-well plate	1	4	0.4~1.6	2~6
6-well plate	2	9.5	0.8~3.2	4~12
35mm dish	2	8	0.8~3.2	4~12
60mm dish	5	20	1.6~6.4	8~24
10cm dish	10	60	3.2~12.8	16~48
125ml flask	30			50~70
1L flask	250			400~600

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Easy and fast procedure

Transfection protocol (35-mm dish):



Preparation of cells

■ Adherent cells

One day before transfection, plate $0.2\text{--}2 \times 10^6$ cells in medium per 35-mm dish. Incubate at 37°C (5% CO₂) overnight. The confluency of cells should be 50–80% before transfection.

■ Suspension cells

Plate 2 ml freshly passaged cells at a density of 5×10^4 /ml to 1×10^6 /ml in a 35-mm dish. The suspension cells should be in log growth phase at the time of transfection. Incubate cells at 37°C (5% CO₂) overnight. The cell number depends on your needs and the cell type to be transfected.

Preparation of Sinofection&DNA complexes

Dilute DNA with appropriate diluents, such as PBS, NaCl solution, glucose solution, DMEM, medium with or without serum, whichever is isotonic, to a concentration of 20 µg/ml (optimization recommended) and a total volume of 250 µl.

Dilute Sinofection of appropriate amount in 250 µl of medium.

Combine the diluted DNA with the diluted Sinofection (total volume = 500 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

Transfection

Add the 500 µl complexes drop-wise to cells and medium. Gently rock the dishes or wells to ensure even distribution.

Incubate cells at 37°C in a CO₂ incubator for 18-48 hours (normally high expression is achieved after 72 hours) prior to protein expression assay.

Transfection protocol (flask):

Transfecting Cells for Protein Expression

This protocol is typically used to transfect DNA into 293 EBNA or CHO cells cultured in flasks.

Use sterile DNA or filter-sterilize DNA before use (re-quantify your DNA after filtration).

All amounts are on a per flask basis for **30 ml cultures** in a 125 ml shake flask; for other formats, see **Table 2. Scaling up or Down Transfections**.

1. Pass 293 cells at 0.3×10^5 cells/ml, shake at 175 rpm. After 72 hours, dilute cells to 1.0×10^6 cells/ml, shake at 175 rpm. Transfect after 4 hours.
2. Pass CHO cells at 0.3×10^5 cells/ml, shake at 175 rpm. After 48 hours, dilute cells to 1.0×10^6 cells/ml, shake at 175 rpm. Transfect after 4 hours.
3. Dilute the required plasmid DNA amount with appropriate diluent, such as PBS with Mg^{2+} , 150mM NaCl, 300mM glucose, DMEM medium with or without serum, whichever is isotonic. Incubate for 5 min at room temperature. Add the required Sinofection volume to the diluted DNA and mix gently to assure a total volume of 1.5~3ml.
4. Incubate the mixture for 10-20 minutes at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.
5. Slowly add 1.5~3ml of DNA-lipid mixture into the 125ml flask containing cells while slowly swirling the flask.
6. Incubate transfected cell cultures at $37^\circ C$, 8% CO_2 on an orbital shaker set to 175 rpm for both 293 cells and CHO cells.

Low cytotoxicity

Sinofection demonstrates very low cytotoxicity which is tested and verified by in-house WST-8 assay for each lot. The WST-8 assay is based on the colorimetric reduction of water soluble tetrazolium (WST) by mitochondrial dehydrogenase

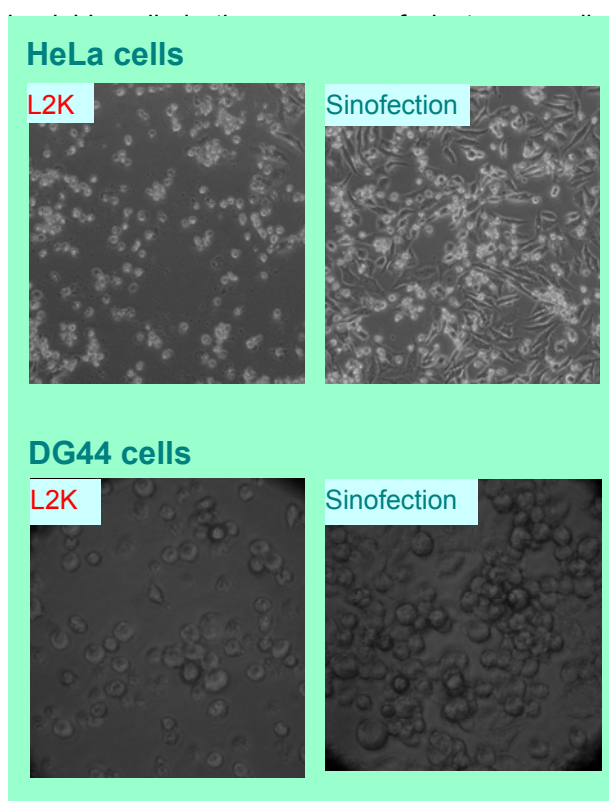


Fig. 3. Microscopy comparison of HeLa cells and DG44 cells transfected with L2K and Sinofection respectively. Transfections were performed following the manufacturer's recommendations.

The absorbance of formazan dye solution is in direct proportion to the number of viable cells. Compared with the prevalent WST-1 method, the sensitivity of WST-8 assay is higher meanwhile the solubility and stability of formazan dye is better.

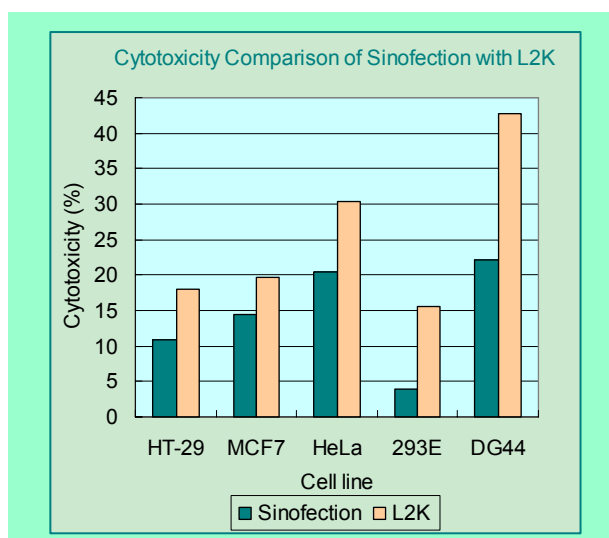


Fig. 4. Cytotoxicity comparison of Sinofection and L2K for 4 cell lines. Transfections were performed following the manufacturer's recommendations.

Quality Control



■ Transfection efficiency:

Sinofection is lot tested against 293E cell line to assure consistency and efficacy. 85% confluent 293E cells in 24-well plate are transfected with 0.6 µg of an rhFc-expression plasmid using 2.8 µl of Sinofection.

■ Sterility

Sinofection is manufactured and packaged in sterile condition and filtrated through 0.22µm sterile filter for the last step.

■ Cytotoxicity

Cytotoxicity of Sinofection is evaluated by in-house WST-8 assay.

Ordering information



Sinofection of various packages

Cat. No.	Pack	Price
	0.75ml	\$80 (250~750 transfections ¹)
	1.5ml	\$150 (500~1500 transfections ¹)
	Bulk	\$150 for transfection ² in 1 L culture

Note:

1. in 24-well plate
2. up to 2.5ml for transfection in 1L culture

Contact and Support

For product information and orders:

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