invitrogen
by life technologies

FreeStyle™ MAX Reagent

Part no. 16447.pps MAN0001311 **Rev. Date** 24 June 2011

Cat. nos.:	Size:	Store at 4°C (do not freeze)
16447-100	1.0 mL	
16447-500	15.0 mL	
16447-750	10 x 15.0 mL	

Description

FreeStyle™ MAX Reagent is a proprietary, animal origin-free formulation for transfecting plasmid DNA into eukaryotic cells that can easily be scaled up to produce large amounts of recombinant proteins. FreeStyle™ MAX Reagent allows the *highest expression levels and transfection rates* with *lowest cytotoxicity* in bio-production applications, and is specifically formulated for use with:

- FreeStyle™ 293-F Cells (suspension human embryonal kidney cells, Cat. no. R790-07) in serum-free FreeStyle™ 293 Expression Medium (Cat. no. 12338-018)
- FreeStyle™ CHO-S Cells (suspension Chinese Hamster Ovary cells, Cat. no. R800-07) in serum-free FreeStyle™ CHO Expression Medium (Cat. no. 12651-014)
- DG44 Cells (DHFR- suspension CHO cells) in CD DG44 Medium with 8 mM L-glutamine and 18 mL/L 10% Pluronic® F-68 (Cat. no. A11000-01)

FreeStyle™ MAX Reagent is intended for use with the FreeStyle™ MAX 293 Expression System (Cat. no. K9000-10) and the FreeStyle™ MAX CHO Expression System (Cat. no. K9000-20).

Important Guidelines for Transfection

- Cultivate FreeStyle™ 293-F and CHO-S Cells, and DG44 Cells in a humidified 37°C, 8% CO_2 environment in suspension on an orbital shaker.
- CHO-S Cells can be transfected in 0.5X Pen/Strep (Cat no. 15140-122), but many other FreeStyle™ MAX transfections are done without antibiotics. Work under sterile conditions and prevent contamination of your DNA.
- Cell-clumping lowers the transfection efficiency; prevent clumping by using the suggested frequent passage schedule and agitation.
- We recommend using OptiPRO™ SFM (1X), liquid (100 mL, Cat. no. 12309-050) to dilute the DNA and lipid before complexing.

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Culturing FreeStyle™ Cells

For routinely culturing *FreeStyle™ 293-F Cells*, shake at 135–155 rpm. Keep cell densities between 0.1 and 3.0×10^6 cells/mL of culture. A cell density above 3.0×10^6 cells/mL will result in a loss of transfection efficiency.

For routinely culturing *FreeStyle™ CHO-S cells*, shake at 120–135 rpm, and keep cell densities between 0.05 and 1.5×10^6 cells/mL of culture. Supplement FreeStyle™ CHO Expression Medium with L-glutamine (Cat. no. 25030-081) to a final concentration of 8 mM. If large numbers of cells are needed, seed cultures at 0.5×10^6 cells/mL and use cells *as soon as* they reach a density of 5×10^6 cells/mL (3–4 days).

Transfecting FreeStyle™ Cells for Protein Expression

Use this procedure to transfect DNA into FreeStyle™ 293-F or CHO-S cells. Use sterile DNA (or filter-sterilize the DNA before use, then re-quantify the 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 **Scaling up or Down Transfections of FreeStyle™ Cells** (page 3).

1. Approximately 24 hours before transfection, pass FreeStyle™ 293-F cells at $6–7 \times 10^5$ cells/mL; shake at 135–155 rpm. Pass FreeStyle™ CHO-S cells at $5–6 \times 10^5$ cells/mL; shake at 120–135 rpm. Culture at 37°C, 8% CO_2.
2. On the day of transfection, the cell density should be $1.2–1.5 \times 10^6$/mL. Dilute the cells to 1×10^6 /mL with growth medium. To ensure optimal transfection, cell viability must be > 95%. Add 30 mL of cells to each flask.
3. Gently invert the tube of FreeStyle™ MAX Reagent 4 times (do not vortex).
4. Dilute 37.5 µg of plasmid DNA into OptiPRO™ SFM to a total volume of 0.6 mL and mix. In a separate tube, dilute 37.5 µL of FreeStyle™ MAX Reagent in OptiPRO™ SFM to a total volume of 0.6 mL. Mix gently by inverting the tube (do not vortex). Immediately add diluted FreeStyle™ MAX Reagent to diluted DNA solution to obtain a total volume of 1.2 mL and mix gently.
5. Incubate the DNA-lipid mixture for 10–20 minutes at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.
6. Slowly add 1.2 mL of DNA-lipid mixture into the 125-mL flask containing cells while slowly swirling the flask.
7. Incubate transfected cell cultures at 37°C, 8% CO_2 on an orbital shaker set to 135 rpm for FreeStyle™ 293-F cells and FreeStyle™ CHO-S cells. There is no need to change or supplement the medium during the first 6–7 days.

Optimizing Protein Expression in FreeStyle™ Cells

- Protein expression can be detected within 4–8 hours of transfection, with maximal protein yield usually 1–7 days post-transfection, depending on the protein expressed.
- When expressing a protein for the first time, perform a time course experiment between days 1–9 post-transfection to identify the peak of protein production, and to monitor cell viability.
- Vary amounts of plasmid DNA and FreeStyle™ MAX Reagent. For 30-mL cultures, try a range of 24–45 µg plasmid DNA and 24–45 µL lipid.
- For secreted IgG protein production, we have observed peak yields at 5–7 days post-transfection.
- To assess transfection efficiency via a GFP-type fluorescent protein, monitor the cultures starting at 24 hours post-transfection.
- For optimizing protein expression while scaling up culture volumes, see **Scaling up or Down Transfections of FreeStyle™ Cells** in the following section**.**

Scaling Up or Down Transfections of FreeStyle™ Cells

To transfect cells in different culture volumes, vary the amounts of FreeStyle™ MAX Reagent, DNA, cells and medium in proportion to the culture volume, as indicated in the following table:

Cell Culture		Dilution	DNA		FreeStyle™ MAX Reagent	
Volume	**Flask**	**Volume**	**Starting Point**	**Optimization Range**	**Starting Point**	**Optimization Range**
30 mL	125 mL	2 × 0.6 mL	37.5 µg	24–45 µg	37.5 µL	24–45 µL
250 mL	1 L	2 × 5 mL	312.5 µg	200–375 µg	312.5 µL	200–375 µL
1 L	3 L	2 × 20 mL	1.25 mg	0.8–1.5 mg	1.25 mL	0.8–1.5 mL

For culture volumes *above 30 mL* further adjustments may be necessary:

- Lower the speed of the orbital shaker if foam is generated. In 1 L cultures, we recommend 70–80 rpm for FreeStyle™ CHO-S Cells, and as close to 135 rpm as possible (without creating foam) for FreeStyle™ 293-F Cells.
- In 1 L cultures, incubate the DNA-lipid mixture for *20 minutes* at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.

Transfecting DG44 Cells to Generate Stable Cell Lines

Use this procedure to transfect linearized DNA into DG44 cells. Use *30-mL cultures* in 125-mL shake flasks; all amounts are given on a per-flask basis.

1. At 48 hours before transfection, pass DG44 cells at 3×10^5 cells/mL; shake at 130–135 rpm at 37°C, 8% CO_2. Culture in CD DG44 Medium (Cat. no. 12610-010) with 8 mM L-glutamine (Cat. no. 25030-081) and 18 mL/L of 10% Pluronic® F-68 (Cat. no. 24040-032).
2. At 24 hours before transfection, again pass DG44 cells at 3×10^5 cells/mL.
3. On the day of transfection, prewarm the CD DG44 Medium (with 8 mM L-glutamine and 18 mL/L of 10% Pluronic® F-68) to 37°C.
4. Count cells (viability must be > 95%). Add 1.5×10^7 cells in a total volume of 30 mL CD DG44 medium to each flask. Place flask in shaker until transfection.
5. Gently invert the tube of FreeStyle™ MAX Reagent 4 times (do not vortex).
6. Add 18 µg of linearized DNA and 15 µL of FreeStyle™ MAX Reagent into 1.2 mL OptiPRO™ SFM (at room temperature) and gently invert to mix.
7. Incubate the DNA-lipid mixture for 10 minutes at room temperature to allow complexes to form. Do not incubate for longer than 20 minutes.
8. Slowly add the 1.2 mL of DNA-lipid mixture into the 125-mL flask containing cells while slowly swirling the flask.
9. Incubate transfected cell cultures at 37°C, 8% CO_2 on an orbital shaker platform rotating at 130–135 rpm.
10. Place cells on selective medium (CD OptiCHO™ Medium, Cat no. 12681-011) 48 hours post-transfection.

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