



## Protein production in mammalian cells



FreeStyle™ 293 Expression System is the way to turn mammalian cells into a protein production system. FreeStyle™ is:

- High-yield
- Scalable
- A complete optimized system

## High protein yield



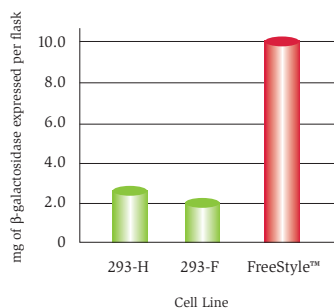
FreeStyle™ 293 Expression System is the answer to the challenge of generating functional protein for downstream applications. Using adherent mammalian cell culture techniques you would have to handle 4 to 5 large T-160 flasks to get the same amount of protein you can now get with one easy-to-handle shaker flask (Figure 1). To get even more protein with less effort you can easily scale up to use larger flasks, spinner flasks or bioreactors.

## Mammalian expression like never before

You've never been able to get such high protein yields from mammalian cells so easily. The FreeStyle™ 293 Expression system is a complete, suspension cell-culture system for generating large amounts of mammalian recombinant protein. Free your lab from the time-consuming, labor-intensive process of seeding, feeding, passing, and maintaining

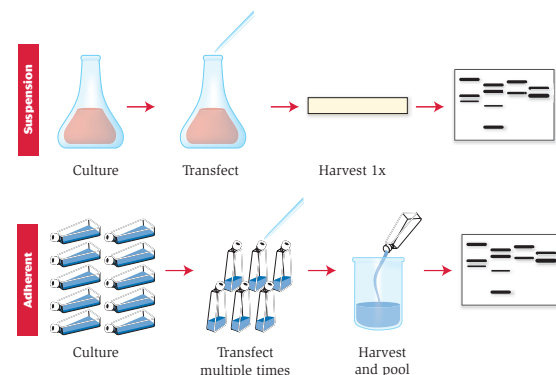
multiple flasks of adherent cells for multiple transfections (Figure 2). FreeStyle's provides a straightforward method to transfect cells in suspension that is so fast and easy you'll shave hours—even days—off traditional adherent transient protein production processes. With FreeStyle™ you'll save time and get higher protein yields than ever before.

Figure 1 - Protein yield per flask



5 x 10<sup>6</sup> 293-F and 293-H cells were plated in T-160 flasks 24 hours prior to transfection such that they were 90% confluent at the time of transfection with Lipofectamine™ 2000. Following transfection the cells were cultured in 25 ml serum containing medium. FreeStyle™ 293-F cells were transfected at a cell density of 1 x 10<sup>6</sup> cells/ml in a 60 ml shaker flask using 293fectin™ and the FreeStyle™ protocol. Protein was measured 48 hours after transfection.

Figure 2 - Save effort using suspension culture



## Serum-free medium eliminates hassles

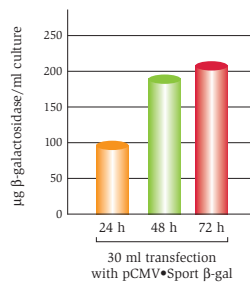
GIBCO™ FreeStyle™ 293 Expression Medium is a specially formulated, defined serum-free medium that supports growth and transfection of FreeStyle™ 293-F cells.

GIBCO™ FreeStyle™ 293 Expression Medium offers you:

- Component definition for consistent performance
- Easier purification and downstream processing
- Increased growth and high protein yields (Figure 3)

Start getting the many benefits of serum-free medium when you perform transient transfections for protein generation by using GIBCO™ FreeStyle™ 293 Expression Medium.

**Figure 3 - Protein produced from a transient FreeStyle™ transfection**



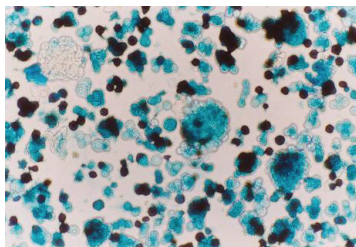
FreeStyle™ 293-F Cells were cultured in 30 ml of FreeStyle™ 293 Expression Medium to a density of  $1 \times 10^6$  cells/ml. Lipid:DNA complexes were prepared using 30 µg of pCMV•SPORT β-gal DNA and 40 µl of 293fectin™. At the timepoints shown, cells were harvested and assayed for level of β-galactosidase protein. An untransfected control was performed in parallel and produced no measurable β-gal activity at any of the time points.

## Cells and reagent working together for high performance

293fectin™ reagent, a new, easy-to-use transfection reagent, is an integral component of the FreeStyle™ System that provides high transfection efficiencies and activities in FreeStyle™ 293-F cells. The 293fectin™ protocol is designed and tested for optimal transfection using suspension 293 cells. More than 98% of the cells in a

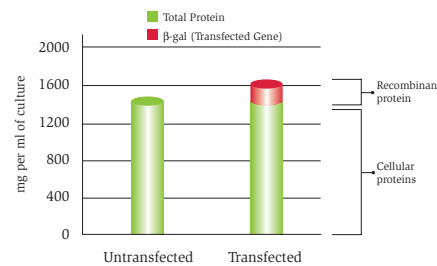
culture are transfected (Figure 4), leading to high protein yields. The system is pre-optimized and high yields are obtained without effects on cell growth (Figure 5). FreeStyle™ 293-F cells, 293fectin™ and GIBCO™ FreeStyle™ 293 Medium work together to give you the most robust production of bioactive protein possible.

**Figure 4 - Transfected FreeStyle™ 293-F cells in suspension**



FreeStyle™ 293-F cells were transfected with pCMV•SPORT β-gal using 293fectin™ and grown with FreeStyle™ 293 Expression Medium in a bioreactor. Cells were stained with X-gal four days following transfection.

**Figure 5 - 8% of protein recovered from a FreeStyle™ production run is recombinant protein**



FreeStyle™ 293-F cells were grown in FreeStyle™ 293 Medium and transiently transfected using 293fectin™ as recommended in the manual. Forty-seven hours post-transfection,  $1 \times 10^6$  cells were harvested and tested for total protein and for β-galactosidase activity. Transfected and untransfected control cells had similar levels of total protein. However, transfected cells expressed 124 µg of β-galactosidase per ml of culture. This represents 8% of the total protein.

## Complete system for transfection and high-yield expression

FreeStyle™ 293 Expression is an optimized system, which simplifies and streamlines production of protein in 293 cells. It contains:

- **293fectin™**, a transfection reagent for high-efficiency transfection of FreeStyle™ 293-F cells.
- **GIBCO™ FreeStyle™ 293 Expression Medium**, a defined serum-free medium manufactured to comply with strict biochemical, performance and regulatory specifications. The medium is a complete formulation capable of supporting FreeStyle™ 293-F cell proliferation and encourages a high level of protein production without additional supplementation.
- **FreeStyle™ 293-F Cells**, a clone of 293 cells with superior handling properties and expression characteristics that is pre-adapted to FreeStyle™ 293 Expression Medium. These cells are mammalian, so expressed proteins will have mammalian post-translational modifications.

FreeStyle™ 293 Expression System also provides Opti-MEM® I for improved DNA:lipid complex formation and a  $\beta$ -galactosidase expression plasmid to test transfection efficiency. The FreeStyle™ 293 Expression System contains enough reagents to perform twenty-five 30-ml transfections, and is scalable for the other transfection volumes that you require.

Every FreeStyle™ System component is also available separately. Contact your account manager for special packaging to meet your needs. Find out more today.

Product	Quantity	Cat. No.
FreeStyle™ 293 Expression System	1 L GIBCO™ FreeStyle™ 293 Expression Medium 1 x 10 <sup>7</sup> FreeStyle™ 293-F Cells (frozen) 1 ml of 293fectin™ 100 ml of Opti-MEM® I 25 µg of pCMV•SPORT $\beta$ -gal	K9000-01
GIBCO™ FreeStyle™ 293 Medium	1 L 6 x 1 L	12338-018 12338-026
293fectin™ Transfection Reagent	1 ml	12347-019
FreeStyle™ 293-F Cells	1 x 10 <sup>7</sup> cells	R790-07



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