



A chemically defined, protein and serum-free medium, which has been formulated without human or animal origin components. CD 293 has been developed for the suspension adaptation and high-density culture of 293 cells.

**Cat. No:**

Liquid	11913-019	1000 mL
AGT™	12529-020	1 X 1 L
	12529-012	1 X 10 L

Without L-glutamine. NOTE: Add L-glutamine before use (see below)

Custom packaging available upon request.

Liquid Storage conditions: 2 to 8°C, in the dark.

AGT Storage conditions: 2 to 8°C or -5 to -20°C, dark and dry.

**Shelf Life**

Refer to product label for expiration date.

**Intended Use**

CD 293 is used to adapt and support the growth and expansion of human embryonic kidney 293 cells and is intended for laboratory research use only.

**Features of CD 293 Media**

- A protein and serum-free formulation designed to support the high-density culture of 293 cells in suspension.
- Formulated without human or animal origin products.
- CD 293 has been formulated without L-glutamine to avoid problems associated with L-glutamine degradation and to maximize shelf life.
- CD 293 supports 293 cellular production of adenovirus and/or glycosylated recombinant protein, comparable in many instances to levels produced in serum-supplemented classical media.
- Supports the large-scale, high-density growth of 293 cells in bioreactors.
- Supports the expansion of transfected 293 cells.
- Supports the high-density suspension culture of HeLa S3 cells.

**Format Features of AGT**

**Advanced Granulation Technology - AGT™**

- CD 293 Medium AGT is easily solubilized.
- CD293 Medium AGT is pH auto-adjusted. When reconstituted per instruction provided below no pH or osmo adjustment is required. Reference Certificate of Analysis for pH & osmo specification.
- CD 293 Medium AGT exhibits comparable cell performance to 1X liquid CD 293 Medium using CD 293 QC assay.
- CD 293 Medium AGT is formulated without L-glutamine

**Introduction**

The 293 cell line was produced by the transformation of human embryonic kidney cells by a sheared adenovirus type 5 DNA (1). The 293 cell line is important as a research tool due to its ability to produce adenovirus and glycosylated human recombinant protein(s).

293 cells have traditionally been grown in two-dimensional monolayer cell cultures with some version of a serum-supplemented complex basal medium. Due to the inclination of 293 cells to aggregate, these cells have not routinely been

grown in suspension. CD 293 is a protein and serum-free medium developed to support the adaptation of adherent-dependent 293 cells to high-density suspension culture (see Adaptation Protocol). The availability of CD 293 represents an enabling technology allowing users to maximize experimental results with suspension cultures while eliminating concerns associated with the use of serum or other materials of human or animal origin.

**Precautions:**

- CD 293 is **not** recommended for **adherent** 293 cell culture.
- The presence of antibiotics in the medium may retard cellular growth.
- **Cells, maintained at levels of 5% CO<sub>2</sub> in air, exhibited a reduced growth rate.**
- Once AGT is reconstituted into a liquid form, performance of the media may vary among cell lines and should be tested in specific applications prior to use. For this reason, AGT shelf life may vary for some specific cell lines applications.

**Instructions for Use**

CD 293 requires supplementation with 4 mM L-glutamine (Cat. No. 25030) or GLUTAMAX™ I (Cat. No. 35050). Addition of a surfactant such as PLURONIC® F68 is not required.

**General Information**

Typically, 293 cells grown in CD 293 demonstrate a doubling time in the range of 17-40 hours. Under our conditions 293 cells cultured in CD 293 have typically achieved cell densities of ~3 x 10<sup>6</sup> cells/mL in shaker or spinner culture (see Figure 1) and ~4 x 10<sup>6</sup> cells/mL in bioreactor culture. Individual culturing and passaging techniques together with inherent cellular heterogeneity may contribute to the demonstration of different results. 293 cells demonstrate a proclivity towards aggregation. Some minor clumping may occur with 293 cells in CD 293. To obtain a complete picture of cellular proliferation, one should vortex to minimize clumps. Cell densities reported here are subsequent to a 45-second vortex at the maximum setting. CD 293 has supported the production of adenovirus.

**Reconstitution Instructions for AGT**

Measure 90% of final volume deionized distilled water. Add CD 293 Medium AGT to water. Mix for 30 minutes or until dissolved completely. Dilute to final volume with water. CD 293 Medium AGT contains sodium bicarbonate – DO NOT ADD. pH/osmo is autoadjusted. Upon reconstitution store at 2-8°C and protect from light. See label or Certificate of Analysis for pH & osmo specification. Sterilize by membrane filtration. Aseptically supplement L-glutamine concentration at time of use.

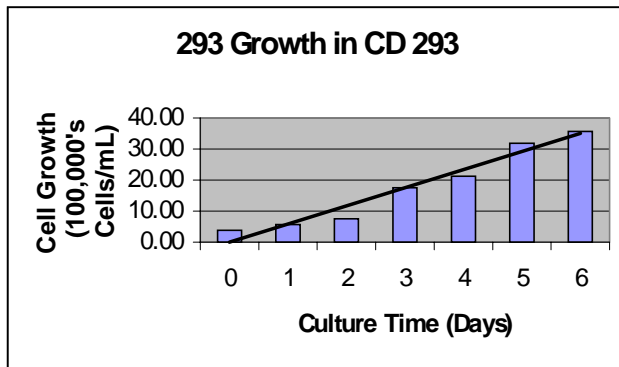


Figure 1. Typical 293 Cell Growth in CD 293 Medium

**Conditions:** 125mL plastic shaker flasks, maximum fill volume of 20mL, initial seeding density 3 x 10<sup>5</sup> cells/mL, 37°C in a humidified atmosphere of 8% CO<sub>2</sub> in air. Figure 1 represents the summation of seventy-four (74) experimental data points.

## Adaptation Protocol for Adapting 293 Cells From an Adherent-Dependent Culture Mode to Suspension Culture

1. If 293 cells are currently being cultured in a serum-supplemented medium, remove as much medium as possible. Displace 293 cells from the flask's surface by rapping the flask sharply against your hand or a protected surface several times. **Do not use trypsin or other proteolytic agents to dislodge cells.**
2. Re-suspend dislodged cells in 5 mL of CD 293. Triturate with a small bore pipette until most cell clumps are dispersed into a single-cell suspension. Count the cells in the presence of 0.4% Trypan Blue solution (Cat. No. 15250 at a 1:9 (v/v) dilution of Trypan Blue solution to cell suspension) in a hemocytometer to determine cell concentration and viability. Automated methods such as the Coulter counter may also be used to determine cell density.
3. Dilute the cells with CD 293 (warmed to 37°C) to a density of  $1.0 \times 10^6$  viable cells/mL. A total volume of 20 mL of the cell solution should be put into each disposable sterile 125-mL plastic Erlenmeyer flask.
4. Incubate the shake flask(s) on a rotary shaker at 125 rpm at 37°C in a humidified, 8% CO<sub>2</sub> in air atmosphere.
5. Determine cell count and viabilities daily. When the viable cell count reaches  $\sim 1.5 \times 10^6$  cells/mL, dilute to  $2.5\text{-}3.0 \times 10^5$  cells/mL with warmed (37°C) CD 293. Continue to dilute to  $2.5\text{-}3.0 \times 10^5$  cells/mL whenever the cell densities are  $\geq 7.5\text{-}10 \times 10^5$  cells/mL.
6. After adaptation to growth in serum-free suspension culture, it is possible to scale-up the cultures in spinner flasks or bioreactors. The appropriate spinner or impeller speed should be individually determined. Under our conditions, the optimum spinner speed was  $\sim 150$  rpm for a simple T-shaped impeller and 70-100 rpm impeller speed in our bioreactor system configuration.

**Caution:** Some spinner apparatus produce significant heat and water-jacketed incubators usually cannot readily equilibrate to temperature variations. Temperatures greater than 40°C are lethal to 293 cells. Ensure a stable environment before placing cells in the incubator.

## Transfection Protocol

**Note:** Complex formation of DNA with transfection reagents such as LIPOFECTAMINE Plus™ (Cat. No. 10964) and LIPOFECTAMINE™ 2000 (Cat. No. 11668) are inhibited by constituents of CD 293. Therefore, these transfection reagents and media should not be used together.

### Prepare DNA-liposome complexes:

1. To each well of a six-well plate, add 1 mL OPTI-MEM® I Reduced Serum Medium (Cat. No. 31985).
2. For each transfection, add 3-4 µg of DNA to each well. Gently swirl to mix.
3. For each transfection, add 10-20 µL of LIPOFECTAMINE™ 2000 to each well. Gently swirl the plate repeatedly to mix the DNA and lipid.
4. Incubate at room temperature for 20-45 minutes to allow DNA-liposome complexes to form. Although the solution may appear somewhat cloudy it will *not* impede the process of transfection.

### While the DNA-liposome complexes are forming, prepare the 293 cells for transfection:

5. Determine the viable cell count of a suspension culture of 293 cells growing in CD 293. Cells must be in *logarithmic growth* prior to transfection for optimal results.
6. Calculate the volume of cell suspension required to plate 293 cells into six-well plates at  $2 \times 10^6$  cells/well. Include two wells ( $4 \times 10^6$  cells) overage. Transfer this volume of cell suspension to a sterile 50 mL centrifuge tube. Bring the total volume to 50 mL with OPTI-MEM I.
7. Pellet cells by centrifuging the tube at 100x g for 4 minutes.

8. Gently aspirate the supernatant, being sure not to disturb the cell pellet.
9. Re-suspend the cell pellet using 10 mL of OPTI-MEM I, triturating to achieve a single cell suspension. Bring the total volume to 50 mL with OPTI-MEM I.
10. Pellet cells by centrifuging the tube at 100x g for 4 minutes. This second wash step is *imperative* for optimal transfection efficiency.
11. Gently aspirate the supernatant, being careful not to disturb the cell pellet.
12. Re-suspend the cell pellet using 200 µL of OPTI-MEM I for every  $2 \times 10^6$  cells contained in the tube. This will yield a solution of  $1 \times 10^7$  cells/mL.
13. Add 200 µL of cell suspension to each well of the six-well plate containing the DNA-liposome complexes. Pipet up and down to mix well.
14. Incubate the plate for 5 hours at 37°C in an 8% CO<sub>2</sub> incubator. There is no need to remove the transfection mixture, or to feed with growth medium.
15. For transient expression, harvest and assay cell extracts or stain cells *in situ* for reporter gene activity at 24 hours after the start of transfection. For stable expression, passage the cells at 24 hours post transfection using the same seeding density or split ratio that is normally used. At 48 hours post transfection, replace the medium on the cells with medium containing the appropriate selective antibiotic (e.g. GENETICIN®). Note: Suspension cells will need to be pelleted by centrifugation (100x g for 4 minutes) and resuspended in medium containing the selective antibiotic.

**Note:** When using different sized tissue culture plates, adjust the amount of DNA, LIPOFECTAMINE™ 2000 and cell concentration in proportion to the difference in surface area of the vessel. One well of a 6 well plate has a surface area of approximately 9.5 cm<sup>2</sup>.

293 cells cultured in CD 293 may grow as 2-10 cell clusters, therefore samples should be vortexed vigorously before passaging or counting. The user must determine optimal vortexing conditions based upon speed, time and viability.

Customers wishing to transfect 293 cells in an anchorage-dependent system using serum-supplemented medium are urged to call the Invitrogen Tech-Line<sup>SM</sup> at the number listed below for an established protocol.

### References:

- 1) Graham, F. L., Smiley, J., Russell, W. C. and R. Nairn (1977) *J. Gen. Virol.* 36, 59.
- 2) Radominski, R., Hassett, R., Dadey, B., Fike, R., Cady, D. & Jayme, D. *Production-Scale Qualification of a Novel Cell Culture Medium Format. BioPharm, Volume 14, Number 7, (July 2001).*
- 3) Radominski, R., Hassett, R., Dadey, B., Fike, R., Cady, D. & Jayme, D. *Advanced Granulation Technology (AGT™) An alternate format for serum-free, chemically-defined and protein-free cell culture media. Volume 36; 33-39, 2001.*

AGT™ is a registered trademark of Invitrogen Corp.

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For further information on this or other GIBCO® products, contact Technical Services at the following:

United States TECH-LINE<sup>SM</sup>: 1 800 955 6288

Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at [www.invitrogen.com](http://www.invitrogen.com)

For research use/further cell culture manufacturing.

CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

April 2007

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