# Avalanche®-PRO Transfection Reagent

**Cat. No. EZT-PROT-1** **Size: 1.5 ml**
**15 ml**
**Store at 4 °C**

## Description

Avalanche®-PRO Transfection Reagent is a proprietary, animal origin-free formulation of lipids and polymers for transfecting plasmid DNA into eukaryotic cells that can easily be scaled up to produce large amounts of recombinant proteins. Avalanche®-PRO Transfection Reagent allows the highest expression levels and transfection rates with lowest cytotoxicity in bio-production applications, and is specifically formulated for use with suspension 293 and CHO derived cells, such as FreeStyle 293-F Cells (suspension human embryonal kidney cells, Life Technologies, Cat. no. R790-07), FreeStyle CHO-S Cells (suspension Chinese Hamster Ovary cells, Life Technologies, Cat. no. R800-07), and DG44 Cells (DHFR- suspension CHO cells).

**Features:**

- Highest recombinant protein yield
- Lesser plasmid DNA and Transfection Reagent needed
- Compatible with multiple CHO media formulations
- Proven Scalability
- Simplest transfection protocol
- Totally animal free

*In brief, Avalanche®-PRO Transfection Reagent is ideal for Biotherapeutic Protein Production*

## Before You Start:

### Important Tips for Optimal Transfection

Optimize reaction conditions for each cell type to ensure successful transfection. The suggestions below generally yield high efficiency transfection using Avalanche®-PRO Transfection Reagent. Before doing formal transfection, please refer to "**Optimizing Protein**

**Expression in suspension 293 or CHO Cells**" at page 3 for optimization on a certain type of cells.

**Cell culture conditions**: Culture cells in the appropriate medium, temperature, CO2 concentration, and shaking speed. For suspension CHO cells, we recommend Freestyle™ CHO Expression Medium (Life Technologies, Cat. No. 12651014) supplemented with L-glutamine (8 mM final concentration). For suspension 293 cells, we recommend Freestyle™ 293 Expression Medium (Life Technologies, Cat. No.12338018). For DG44 Cells, we recommend CD DG44 Medium (Life Technologies, Cat. no. 12610-010) with 8 mM L-glutamine (Life Technologies, Cat. no. 25030-081) and 18 mL/L 10% Pluronic® F-68 (Life Technologies, Cat. no. 24040-032). Alternative media formulations may also be compatible with transfection. There is no need to perform a medium change to remove the transfection complexes. Medium additives or supplements can be added 24 hours post-transfection. Cultivate Cells in a humidified 37 °C, 8% $CO_2$ environment in suspension on an orbital shaker. For routinely culturing suspension CHO cells, shake at 120–135 rpm; for routinely culturing suspension 293 cells, shake at 135–155 rpm.



**Cell density:** For routinely culturing suspension CHO cells, keep cell densities between 0.05 and 1.5 x$10^6$ cells/ml of culture. If large numbers of cells are needed, seed cultures at 0.5 x$10^6$ cells/ml and use cells as soon as they reach a density of 5 x$10^6$ cells/ml (3–4 days). For routinely culturing suspension 293 Cells, keep cell densities between 0.1 and 3.0 x$10^6$ cells/ml of culture. A cell density above 3.0 x$10^6$ cells/ml will result in a loss of transfection efficiency. Determine the optimal cell density at the time of transfection for each cell type to maximize transfection efficiency. Typically, a cell density of 0.5–1.0 × $10^6$ cells/ml is desired at the time of transfection. Cells should be actively dividing at the time of transfection. Cell-clumping lowers the transfection efficiency; prevent clumping by using the suggested frequent passage schedule and agitation.

**DNA purity:** Prepare high-quality plasmid DNA at 0.5–5 µg/µl in deionized water or TE buffer. Make sure the plasmids are sterile, endotoxin-free, and have A260/280 absorbance ratio of 1.8–2.0.

**Complex formation conditions:** We recommend using OptiPRO SFM (1X), liquid (100 ml, Life Technologies, Cat. no. 12309-050), or Opti-MEM® I Reduced Serum Medium (Cat# 31985-070, Life Technologies) to make complex.

**Presence of antibiotics:** Antibiotics could be added to cell culture medium 24 hours after transfection at the final concentration of 0.1-1 X. DO NOT use antibiotics during the DNA/Reagent complex formation, or at the time of transfection.

## Transfecting Suspension 293 or CHO Cells for Protein Expression

Use the following procedures to transfect DNA into suspension 293 or CHO-S cells. All amounts are on a per-flask basis for 30-ml cultures in a 125-ml Erlenmeyer shake flask; for other formats, see **Scaling up or Down Transfections of Suspension 293 or CHO Cells** (page 4).



1. Maintain cells 18–24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection.
2. On the day of transfection, dilute the cells to 1 x $10^6$ /ml with growth medium. To ensure optimal transfection, cell viability must be > 95%. Add 30 ml of cells to each flasks.
3. Warm Avalanche®-PRO Transfection Reagents to room temperature and vortex gently before using.
4. Dilute 15.0 µg of plasmid DNA into OptiPRO SFM or Opti-MEM® to a total volume of 3 ml and mix gently. Gently vortex the tube of Avalanche®-PRO Transfection Reagent, and add the Reagent (actual optimal amount needs to be determined by the next paragraph "**Optimizing Protein Expression in suspension 293 or CHO Cells**") to the above diluted DNA solution, and vortex immediately and gently.
5. Incubate the DNA-Reagent mixture for 15-30 minutes at room temperature to allow complexes to form.
6. Slowly add the whole DNA-Reagent 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 suspension 293 cells and suspension CHO cells. There is no need to change or supplement the medium during the first 6–7 days.

## Optimizing Protein Expression in suspension 293 or CHO Cells

- When expressing a protein for the first time on a specific type of cells or a certain type of cells from different sources or passages, vary amounts of Avalanche®-PRO Transfection Reagent to achieve maximum protein production. For 30-ml cultures, using 15 µg of DNA, try 8.0 µl, 12, 18µl , and 24 µl of Transfection Reagent to find out the Optimal Amount for best transfection. Also it is necessary to perform a time course experiment between day1–9 post-transfection to identify the peak of protein production, and to monitor cell viability.
- 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.
- For secreted IgG protein production, peak yields are typically obtained 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 suspension Cells in the following section.

## Scaling Up or Down Transfections of suspension 293 or CHO Cells



After determining the Optimal Amount of Avalanche®-PRO for best transfection in 30 ml volume for a certain type of cells as suggested in page 3 "**Optimizing Protein Expression in suspension 293 or CHO Cells**", use the multiplication factors in the following form to determine the Avalanche®-PRO amount needed for your new culture volumes.

| Cell Culture | | Multiplication factor | Dilution | DNA | Avalanche®-PRO |
|---|---|---|---|---|---|
| Volume | Flask | | Volume | µg | µl |
| 30 ml | 125 ml | 1 | 3 ml | 15.0 µg | Optimal Amount (30 ml) x 1 |
| 250 ml | 1 L | 8.3 | 25 ml | 125.0 µg | Optimal Amount (30 ml) x 8.3 |
| 1 L | 3 L | 33.3 | 100 ml | 500.0 µg | Optimal Amount (30 ml) x 33.3 |

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 suspension CHO Cells, and as close to 135 rpm as possible (without creating foam) for suspension 293 Cells.

## Transfecting DG44 Cells to Generate Stable Cell Lines

Use the following procedures 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 x $10^5$ cells/ml; shake at 130–135 rpm at 37 °C, 8% CO2. Culture in CD DG44 Medium with 8 mM L-glutamine and 18 ml/L of 10% Pluronic® F-68.
2. At 24 hours before transfection, again pass DG44 cells at 3 x $10^5$ cells/ml.
3. On the day of transfection, pre-warm 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 x $10^7$ cells in a total volume of 30 ml CD DG44 medium to each flask. Place flask in shaker until transfection.
5. Add 18 µg of linearized DNA into 3.0 ml OptiPRO SFM or Opti-MEM® (at room temperature) and gently vertex

6. Gently vertex Avalanche®-PRO Transfection Reagent, add 12 µl - 30 µl into the above DNA solution, and vortex immediately and gently. Incubate the DNA-Reagent mixture for 15 minutes at room temperature to allow complexes to form.
7. Slowly add the 3.0 ml of DNA-Reagent mixture into the 125-ml flask containing cells while slowly swirling the flask.
8. Incubate transfected cell cultures at 37 °C, 8% $CO_2$ on an orbital shaker platform rotating at 130–135 rpm.
9. Place cells on selective medium (CD OptiCHO Medium, Cat no. 12681-011) 48 hours post-transfection.



**Intended Use:**

All Avalanche® Series Transfection Reagents are for research use only, not intended for any animal or human therapeutic or diagnostic use.



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