

## Product Information

### EX-CELL® CD HEK293 Viral Vector Medium

Catalog Number **14385C**  
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

EX-CELL CD HEK293 Viral Vector Medium is an animal origin-free, chemically defined medium compatible with PEI transfection reagents for high titer viral vector production. This medium is designed for use as a platform in conjunction with VirusExpress™ 293T Lentiviral Production Cells (Catalog Number VP001). EX-CELL CD HEK293 Viral Vector Medium has been formulated to support high suspension cell densities with minimal clumping, a high PEI transient transfection efficiency, and high viral titers in shake flask and stirred tank formats. Viral production using the VirusExpress platform is a scalable suspension process that does not require a media exchange.

EX-CELL CD HEK293 Viral Vector Medium supports high viable cell densities (VCD) of VirusExpress 293T Lentiviral Production Cells.

- Peak VCD:  $1.0\text{--}1.2 \times 10^7$  viable cells/mL
- VCD 48 hours post-transfection (with lentiviral packaging and transfer plasmids):  
 $3.5\text{--}4.5 \times 10^6$  cells/mL, cell viability >90%
- Transfection efficiency:  
35% at 24 hours post-transfection  
80% at 48 hours post-transfection  
(as measured by GFP expression when transfecting using a third-generation packaging system)
- Lentivirus functional titer:  
 $\geq 2.0 \times 10^7$  transducing units (TU)/mL using GFP transgene at scales ranging from 30 mL shake flask to 40 L stirred tank single use bioreactor

See Figures 1-2 for performance data.

### Precautions and Disclaimer

For Research or Further Manufacturing Uses Only-Not Intended for Direct Use in Humans or Animals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

EX-CELL CD HEK293 Viral Vector Medium is formulated without L-glutamine. Add L-glutamine (200 mM, Catalog Number 59202C) to a final concentration of 6 mM using aseptic supplementation technique.

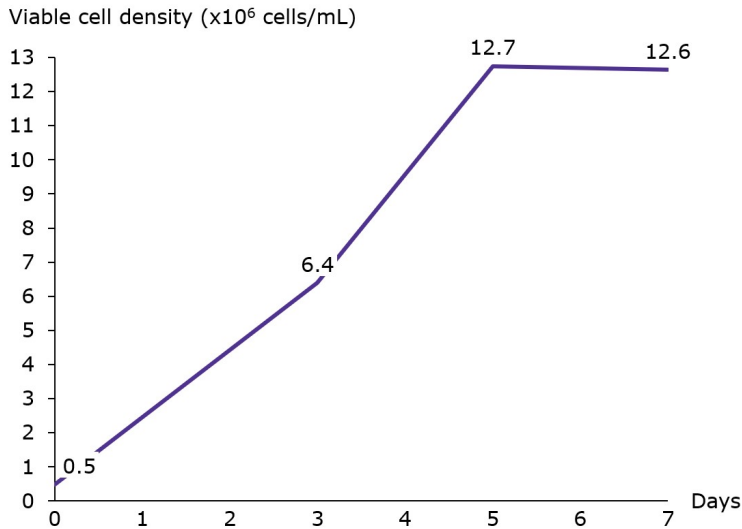
### Components

This medium is chemically defined, animal component-free, and does not contain L-glutamine or antibiotics.

### Storage/Stability

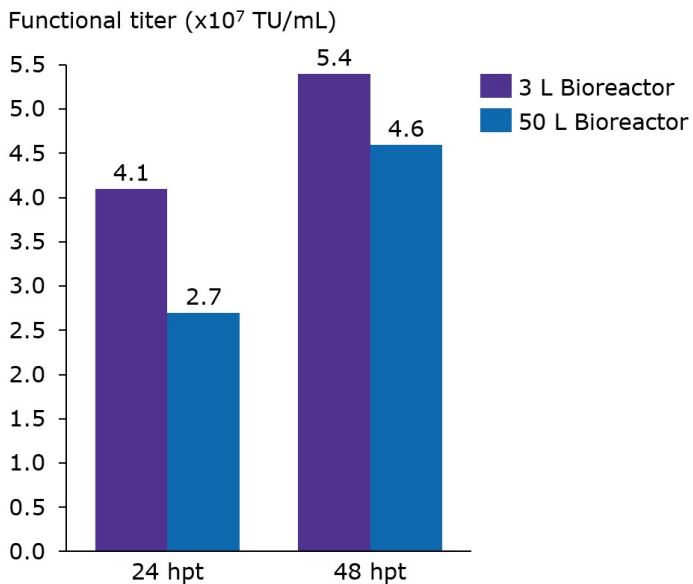
Store medium at 2–8 °C and protected from light. Medium is stable when stored correctly for up to one year. Do not use if medium is cloudy or contains precipitates. Use aseptic technique when handling or supplementing this medium.

**Figure 1.**  
**Viable cell density study**



VirusExpress 293T Lentiviral Production Cells (VP001) were inoculated in duplicate 30 mL cultures in 125 mL shake flasks at  $5.0 \times 10^5$  viable cells/mL. Cells were fed 4 mM L-glutamine and 3 g/L glucose on days 3 and 5.

**Figure 2.**  
**Lentiviral titer in bioreactors**



VirusExpress 293T Lentiviral Production Cells (VP001) were cultured in EX-CELL CD HEK293 Viral Vector Medium in a shake flask seed train. 3 L Mobius® bioreactors were inoculated at pre-transfection density. 50 L Mobius bioreactors were inoculated at 10 L, cultured for approximately 3 days, and diluted to pre-transfection density at approximately 40 L working volume. 24 hours post-inoculation (3 L) or post-dilution (50 L), cells were transfected with lentiviral packaging plasmids and a GFP transfer plasmid using PEI. Crude titers for harvests at 24- and 48-hours post-transfection (hpt) were determined using an HT1080 cell-based functional assay. VirusExpress 293T Lentiviral Production Cells grown and transfected in EX-CELL CD HEK293 Viral Vector Medium maintain high titers when scaled up in bioreactors.

## Frequently Asked Questions

1. **What shaker flasks do you recommended for culturing using EX-CELL CD HEK293 Viral Vector Medium?**

We recommend using a baffled shaker flask for suspension culture to reduce cell clumping at 135 rpm (25 mm orbital diameter)

2. **Do I need to add an anticlumping or antifoam reagent when culturing 293T cells in EX-CELL CD HEK293 Viral Vector Medium?**

EX-CELL CD HEK293 Viral Vector Medium does not require addition of anticlumping reagents or antifoam reagents.

3. **Can I culture VirusExpress 293T Lentiviral Production Cells in EX-CELL CD HEK293 Viral Vector Medium using penicillin/ streptomycin?**

We do not recommend the use of antibiotics for routine culture of HEK293T cells using this medium.

4. **Do VirusExpress 293T Lentiviral Production Cells cultured in EX-CELL CD HEK293 Viral Vector Medium require a medium exchange during lentiviral production?**

The VirusExpress platform supports a suspension process that does not require a post-transfection medium exchange or cell culture feed.

5. **What is the suggested cell density when passaging a suspension culture of VirusExpress 293T Lentiviral Production cells in EX-CELL CD HEK293 Viral Vector Medium?**

We recommend inoculating cultures at  $5.0 \times 10^5$  viable cells/mL for 3-day cultures or  $3.0 \times 10^5$  viable cells/mL for 4-day cultures.

6. **What is the suggested cell density when seeding VirusExpress 293T Lentiviral Production Cells in EX-CELL CD HEK293 Viral Vector Medium prior to transient PEI transfection for lentivirus production?**

We recommend seeding at  $1.2 \times 10^6$  viable cells/mL 18 to 24 hours before transient transfection. Targeted cell density on the day of transfection is  $2.4\text{--}3.5 \times 10^6$  viable cells/mL and viability >95%.

7. **How I can improve the accuracy of cell counts of cultures using EX-CELL CD HEK293 Viral Vector Medium?**

Because cells may grow in small clusters, we recommend using Accumax™ solution (Catalog Number A7089) to improve cell counting. Collect a 600  $\mu$ l cell sample, add an equal volume of Accumax, and mix gently. Incubate tubes at 37 °C on a shaker platform for 30 minutes. Pipette the samples 5–6 times to break up remaining cell aggregates and transfer the volume required for automated cell counter measurement.

8. **What is the recommended plasmid DNA/PEI ratio for transfection of VirusExpress 293T Lentiviral Production Cells cultured in EX-CELL CD HEK293 Viral Vector Medium?**

We recommended using a plasmid DNA/PEI ratio of 1:2.

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