

Certificate of Analysis

HEK 293 Tet-On® 3G Cell Line

Catalog No.

631185 (Not sold separately)

Lot Number

Specified on product label.

Description

HEK 293 Tet-On 3G is a transformed human embryonic kidney-derived cell line that expresses the tetracycline (Tet)-regulated transactivator Tet-On 3G (Zhou et al. 2006; Loew et al. 2010; Wang et al. 2008). Inducible expression of any gene can be achieved by transfecting or transducing this cell line with a vector containing your gene of interest under the control of a tetracycline-responsive promoter. Expression is induced by the addition of doxycycline (Dox) to the culture medium.

Package Contents

- 1 ml HEK 293 Tet-On 3G Cell Line (2×10^6 cells/tube)

Storage Conditions

- Store cells in liquid nitrogen (-196°C) or a -150°C freezer

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Medium

- Cell Freezing Medium-DMSO 1x (Sigma-Aldrich Co., Cat. No. C6164)

Shipping Conditions

- Dry ice (-70°C)

Product Documents

Documents for our products are available for download at takarabio.com/manuals

The following documents apply to this product:

- Tet-On 3G Inducible Gene Expression System User Manual (PT5148-1)
- Tet Cell Lines Protocol-at-a-Glance (PT3001-2)

Cell Type Information

HEK 293 Tet-On 3G is a transformed human embryonic kidney (HEK)-derived cell line stably transfected with pEF1 α -Tet3G. This cell line is G418-resistant.

Recommended Cell Culture Medium

Grow the cells in 90% Eagle Minimum Essential Medium (alpha modification), 10% Tet System Approved Fetal Bovine Serum (FBS), 4 mM L-glutamine, 100 $\mu\text{g/ml}$ G418, 100 units/ml penicillin G sodium, and 100 $\mu\text{g/ml}$ streptomycin sulfate, in the presence of 5% CO_2 .

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Additional Notes

For HEK 293-based cell lines, we recommend using collagen-coated plates or flasks for culturing for efficient recovery of frozen stocks. Culture vessels coated with compounds other than collagen may also provide suitable growth substrates for HEK 293-based cell lines; however, only collagen-coated plates have been tested at Clontech. The cells may be cultured on non-coated flasks/dishes after recovery; however, if adherence is poor, we recommend using collagen-coated vessels for all culturing purposes.

HEK 293 Tet-On 3G cells are especially sensitive to hygromycin. To titrate hygromycin stocks for the optimal concentration, test a concentration range with a midpoint of 25 µg/ml.

HEK 293 Tet-On 3G cells can also be grown in DMEM with the same supplements listed above.

References

Loew, R., Heinz, N., Hampf, M., Bujard, H. & Gossen, M. Improved Tet-responsive promoters with minimized background expression. *BMC Biotechnol.* **10**, 81 (2010).

Wang, R., Liang, J., Jiang, H., Qin, L.-J. & Yang, H.-T. Promoter-dependent EGFP expression during embryonic stem cell propagation and differentiation. *Stem Cells Dev.* **17**, 279–89 (2008).

Zhou, X., Vink, M., Klaver, B., Berkhout, B. & Das, A. T. Optimization of the Tet-On system for regulated gene expression through viral evolution. *Gene Ther.* **13**, 1382–90 (2006).

Quality Control Data

Functional Tests

HEK 293 Tet-On 3G cells were transiently transfected with pTRE3G-Luc. Luciferase activity in the presence and absence of doxycycline (Cat. No. 631311) was measured 48 hr later as described in the Tet-On 3G Inducible Gene Expression System User Manual. Induction was observed to be at least (500-fold) when cells were grown in medium containing our Tet System Approved FBS.

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of *Mycoplasma* contamination.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

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STATEMENT 42

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent # 8383364, # 9181556, European patents EP # 1954811, #2352833 and corresponding patent claims outside these regions which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to any data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Takara Bio USA, Inc. is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems.

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