9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

14-703ACL: TET-ON Leeporter™ GFP Reporter-HEK293 Cell Line

Application : Functional Assay

Description

The TET-ON Leeporter™ GFP Reporter cell line is a stably transfected HEK293 cell line, which expresses enhanced green fluorescent protein (eGFP) reporter gene under the transcriptional control of the Tetracycline-inducible promoter. So the GFP expression can be turned on by treating the cell line with doxycycline which can regulate the level of GFP expression in the cells, while the resting cells do not express GFP. The cell line is designed to analyze by fluorescence microscopy and flow cytometry. The GFP expression in the cell line by doxycycline is shown in Figures 1 and 2.

Product Info

Amount :	1 Vial
Content :	Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Storage condition :	Immediately upon receipt, store in liquid nitrogen.

Application Note

Application:

- Monitor the tetracycline (doxycycline)-controlled GFP expression.
- Tetracycline activity.

Culture conditions:

Cells should be grown at 37°C with 5% CO_2 using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin (Note: Puromycin can be omitted during the reporter cell assays).

It is recommended to quickly thaw the frozen cells upon receipt or from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Puromycin, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin, transfer resuspended cells to T25 flask and culture in 37°C-CO_2 incubator.

Leave the T25 flask in the incubator for 1~3 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are over 90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence.

To passage the cells, detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cells suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.

Functional validation:

9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

A. Response of TET-ON Leeporter™ GFP reporter – HEK293 cells to Doxycycline.

1. Harvest TET-ON Leeporter™ GFP reporter – HEK293 cells and seed cells into a tissue culture plate (e.g. 96-well plate in 100 ul of growth medium at 5 x 10^4 cells/well, 24-well plate in 500 ul of growth medium at 2.5 x 10^5 cells/well, 12-well plate in 1 ml of growth medium at 5 x 10^5 cells/well, or 6-well plate in 2 ml of growh medium at 1 x 10^6 cells/well.

2. Incubate cells at 37°C in a CO_2 incubator for overnight.

3. The next day, stimulate cells with different concentrations of doxycycline.

4. Incubate at 37°C in a CO_2 incubator for 16-24 hours.

5.Analyze cells through fluorescence microscopy or flow cytometry.

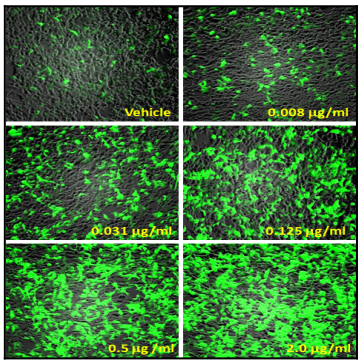

Fig-1: Induction of GFP expression in TET-ON Leeporter™ GFP reporter-HEK293 cells was analyzed by fluorescence microscopy. Doxycycline was treated at different concentrations as noted.

9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

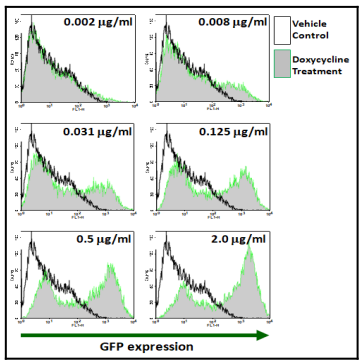

Fig-2: Induction of GFP expression in TET-ON Leeporter™ GFP reporter-HEK293 cells was analyzed by fluorescence microscopy. Doxycycline was treated at different concentrations as noted. Vehicle control (Black line, empty); Doxycycline treatment (Green line, grey-filled).