HeLa-GFP inducible expression stable cell line manual

Catalog Number	Amount	Storage
SC036	1 vial of cells (2 x 10^6 cells) in 80% DMEM, 10% FBS, 10% DMSO	Liquid nitrogen

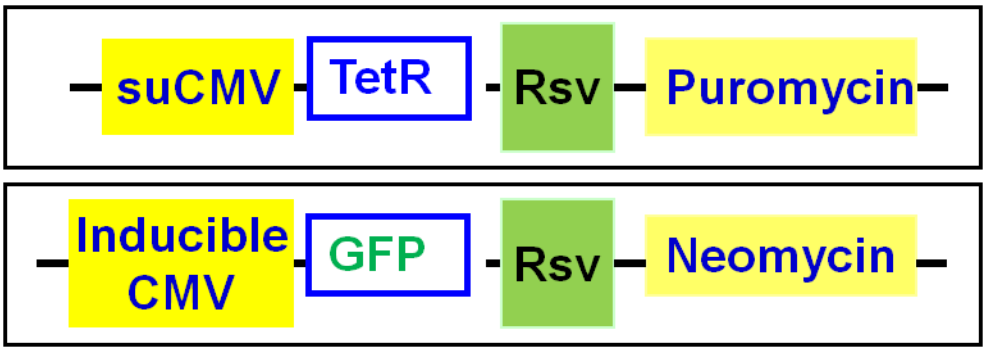
Product Description:

HeLa cell line was derived from human cervical cancer cells. The cell line is remarkably durable, can divide for an unlimited number of times in a laboratory cell culture as long as the fundamental cell survival conditions are met.

This stable cell line is derived from HeLa cells. It constitutively expresses the repressor protein (TetR). The codon optimized **GFP** inducible expression lentivirus was transduced into the HeLa-TetR cell line. The GFP is expressed under the **Tetracycline inducible suCMV promoter**. The repressor (TetR) protein stops/represses the GFP's expression. Therefore, the GFP is not expressed until the tetracycline or doxycycline (inducer) is added into cell culture.

The GFP expression cassette contains neomycin marker expressed under RSV promoter. And the TetR repressor cassette contains a puromycin marker. As a result, the cells demonstrate both neomycin and puromycin resistance.

The following two expression cassettes were integrated into this stable cell line's genome.



Each cell line demonstrates no GFP signal (or weak GFP if there is a leaking expression) before adding tetracycline, and shows strong GFP signal after adding the inducer, tetracycline.

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Culture procedures:

1. Thaw the frozen vial of cells quickly in a 37°C water bath (1-3min), decontaminate the outside of the vial with 70% ethanol.
2. Transfer the entire content of the cryovial into a T-75 cm^2 flask containing 15 ml of pre-warmed complete medium. Incubate the cells overnight in a 37°C incubator, 5% CO_2.
3. The following day, replace the medium with 15 ml of pre-warmed, complete medium (**Optional**: add final 100 µg/ml Neomycin and final 1 µg/ml puromycin in medium).
4. Incubate the cells and monitor cell density.
5. Passage cells (1:10 dilution) when the culture reaches 80-90% confluency.
6. For GFP expression, add tetracycline (or doxycycline) to cell culture at final concentration of 1.0 µg/ml. The GFP expression will be peak at 48 hours post induction.
7. You can visualize GFP signal under a fluorescent microscope (with filter setting: Ex545nm /Em620nm).
8. For cell storage: freeze cells at a density of 3 x 10^6 cells/ml using 90% complete medium with 10% DMSO.

Complete medium:

D-MEM (high glucose)
2mM L-glutamine
10% Fetal Bovine Serum (FBS)*
0.1 mM MEM Non-Essential Amino Acids (NEAA)
1% Pen-strep
10 µg/ml Blasticidin (optional)
5.0 µg/ml of Puromycin (optional)

*: Note: it is better to use Tetracycline free FBS to control the basal expression.

Quality Control:

Each vial contains more than 2 x 10^6 cells with >95% viability before freezing. Cells are tested to be free of bacteria, viruses, mycoplasma.

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