

Certificate of Analysis

CellSensor® T-REx™ FOXO3 DBE-*b/a* HeLa Cell Line, 1 Vial



Combo Kit Part Number: K1468 denotes cells + documentation

Part Number: K1428 Cell Part Number

Lot Number: 1583251

Immediate Storage: Liquid nitrogen or thaw for immediate use.

Shipping Conditions: dry ice overnight (or liquid N₂ followed by dry ice overnight)

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Description:

The CellSensor® T-REx™ FOXO3 DBE-*b/a* HeLa Cell Line was engineered by lentiviral transduction of HeLa cervical cancer cells with a FOXO3 response element driving beta-lactamase expression (DBE-*b/a*), along with a tetracycline repressor and tetracycline (or the tetracycline analog doxycycline)-inducible FOXO3 constructs. Addition of doxycycline to these cells allows for FOXO3 transcription factor expression and subsequent beta-lactamase expression, which in turn can be down-regulated by insulin-mediated activation of the PI3K/AKT signaling pathway which leads to phosphorylation and inactivation of FOXO3 and concomitant suppression of beta-lactamase expression. This cell line has been tested for robust assay performance by assessing a variety of assay parameters, including: cell plating number, DMSO tolerance, stimulation time, and substrate loading time. Assay validation was performed using serial dilutions of insulin to antagonize doxycycline-induced FOXO3 activity. Additional testing data using RNAi and alternate stimuli are also available.

Quantity of Cells: ~ 2,000,000 cells/vial at 2,000,000 cells/mL

Harvest Density:

This lot of cells was harvested at a density of 67,000 cells/cm² for the functional QC testing. A similar harvest density is recommended for your experiments.

Passage Number: 16

Cell Phenotype: Epithelial

Growth Properties: Adherent

Antibiotic Selection:

Blasticidin, 5 µg/mL, Hygromycin B 125 µg/mL, Zeocin™ 75 µg/mL

Storage and Handling:

After shipment either thaw for immediate use or store in liquid nitrogen. **Cells stored for more than 1 day at -80°C quickly lose viability.** If properly banked, cells are guaranteed for six months in liquid nitrogen.

Storage Buffer:

Recovery™ Cell Culture Freezing Medium (Invitrogen #12648-010).

Biosafety Level: 2

Instrument Settings:

The CellSensor® T-REx™ FOXO3 DBE-*b/a* HeLa Cell Line requires a fluorescence plate reader capable of doing FRET and filters in the **excitation filter at 360 nm and an emission filter at 485 nm** range. When used with the LiveBLAzer™ or ToxBLAzer™ substrate an instrument capable of bottom reads is required. For instrument specific set-up information go to www.invitrogen.com/instrumentsetup.

Protocol or Product Information Sheet:

Visit www.lifetechnologies.com, search on the cell or kit part number and select manuals.

QUALITY ASSURANCE

Lot Specific Testing

Mycoplasma: Negative

Functional Testing:

Each lot of the CellSensor® T-REx™ FOXO3 DBE-*b/a* HeLa Cell Line is tested for performance using the LiveBLAzer™ substrate. **Pass**

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Tony Goossens, Engineer II, QA/QC

Date: 05/Feb/2015

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For questions, please contact our Technical Support Team

N. Am Ph#: 800-955-6288 or INTL Ph#: 760-603-7200 Select option 5, ext. 40266 Email: drugdiscoverytech@lifetech.com