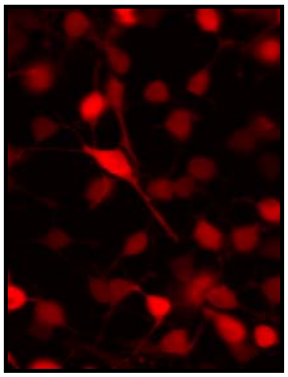
REF: P20305

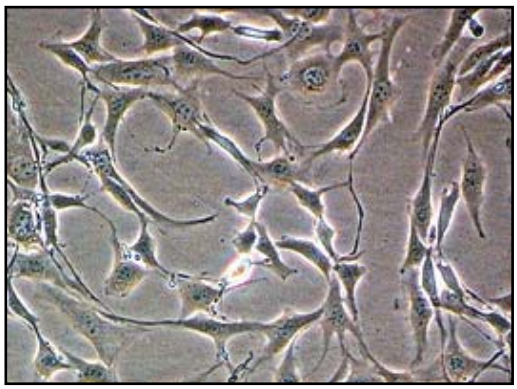
VAMPIRO™ CELL LINES

RED FLUORESCENT NIH/3T3 CELL LINE



Product Name:	VAMPIRO™ – 3T3/NIH Cell line
Catalog Number:	P20305
Cell Type:	3T3 Swiss albino mouse fibroblasts
Fluorescent Protein:	turboFP602 (Evrogen)
Format:	3 x 10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

A novel red fluorescent NIH/3T3 cell line has been developed through stable transfection with Evrogen TurboFP602. This cell line expresses red fluorescent protein gene sequences as free cytoplasmatic proteins.



tGFP-3T3 Cell line is stably transfected clonal cell line that is ready to use in cell-based assay applications. This stably transfected clonal cell line provides consistent levels of expression, which helps simplify the interpretation of results. This cell line is intended to be used for transfection studies with DNA viruses and as assay system for transformation studies; cells have a high sensitivity to contact inhibition.

About NIH/3T3

This cell line was established from disaggregated Swiss albino mouse embryos in 1962. They are fibroblasts growing adherently as monolayer with contact inhibition. Swiss 3T3 cells are inhibited by temazepam and other benzodiazepines. The original cells are extremely contact inhibited, although the cell line is no longer inhibited. 3T3 cells are sensitive to sarcoma virus focus formation, as well as leukemia virus.

3T3 cells are often used in the cultivation of keratinocytes, with the 3T3 cells secreting growth factors favourable to these kinds of cells.

Use Restriction

This product contains a proprietary nucleic acid coding for a proprietary fluorescent protein intended to be used for research purposes only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product, or to use the gene or protein other than for non-commercial research, including use for validation or screening compounds. For information on commercial licensing, contact Licensing Department, Evrogen JSC, email: license@evrogen.com.

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About TurboFP602

TurboFP602 protein is a red shifted variant of the red fluorescent protein TurboRFP from sea anemone Entacmaea quadricolor [Merzlyak et al., 2007].

TurboFP602 posseses true-red fluorescence (with excitation/emission maxima at 574/602 nm, respectively), optimal for detection via most popular filter sets, and is easily distinguished from background signals. TurboFP602 exhibits fast maturation and high pH stability.

Quality Control

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.

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CELL CULTURE INSTRUCTIONS

A. Complete Growth medium

DMEM 10% FBS, 3 μg/ml Puromycin

B. Set up culture after receiving

1. Decontaminate the external surfaces of medium and medium supplements with 70% ethanol.

2. Prepare coated flask (T-75 flask is recommended). Add 9 ml of DMEM and then add 1 ml of FBS (without selection antibiotic). Leave the flask in incubator minimum one hour at 37ºC incubator.

3. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol. Remove the cap, being careful not to touch the interior threads with fingers.

4. Dispense the contents of the vial using 1 ml eppendorf pipette and gently resuspend the contents of the vial in T75 flask containing pre-warmed complete growth media.

5. Place the flask to the incubator.

6. For best result, do not disturb the culture for 16 hours after the culture has been initiated. Change the growth medium (including the selection antibiotic) the next day to remove the DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.

2. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

3. Subculture the cells when they are over 90% confluent.

4. Incubate cells with 1 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 1ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.

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