ESSEN BioSCIENCE

PRODUCT DATA SHEET

CellPlayer™ CytoLight Red (Lenti, EF-1 alpha, bleo)

Essen BioScience Catalog Number: 4484

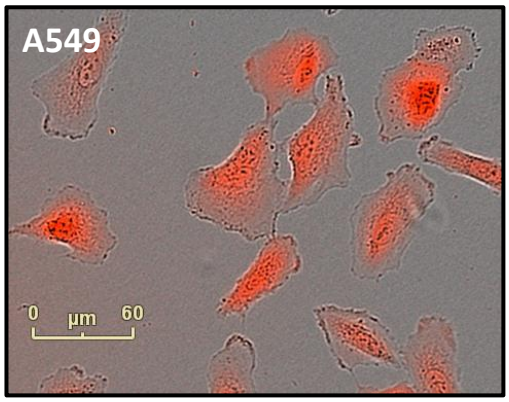
Background

Third generation lentiviral-based vectors are commonly used to transfer genetic information to cells for gene therapy and/or research purposes. The Essen BioScience CellPlayer™ lentiviral-based reagents have been specially designed to efficiently transduce multiple cell types and provide homogenous expression of fluorescent protein across a population of primary or immortalized, dividing or non-dividing cells with low toxicity. Our extensive validation experiments have shown that expression of nuclear restricted RFP does not negatively alter functional cell biology (e.g. morphology, proliferation, migration, and differentiation). These reagents can be used either transiently or to generate stable cell populations or clones using bleomycin selection. The Essen CellPlayer™ lentiviral-based fluorescent protein reagents are particularly suited for use with the IncuCyte ZOOM™ live-cell imaging system.

Virus Description

3rd generation HIV-based, VSV-G pseudotyped lentiviral particles encoding a Red Fluorescent Protein.

Promoter: EF-1 alpha
Selectable Marker: Bleomycin
Spectral Properties: Ex (max): 588 nm; Em (max): 633 nm



Presentation

Lot #:
Viral Titer:
Volume: 0.6 mL

Storage

Lentivirus is stable for at least 3 months from date of receipt when stored at -80°C. After thawing, place immediately on ice and freeze in working aliquots at -80°C**. Additional freeze/thaw cycles may result in decreased viral titers and sub-optimal transduction efficiencies.**

Additional Materials

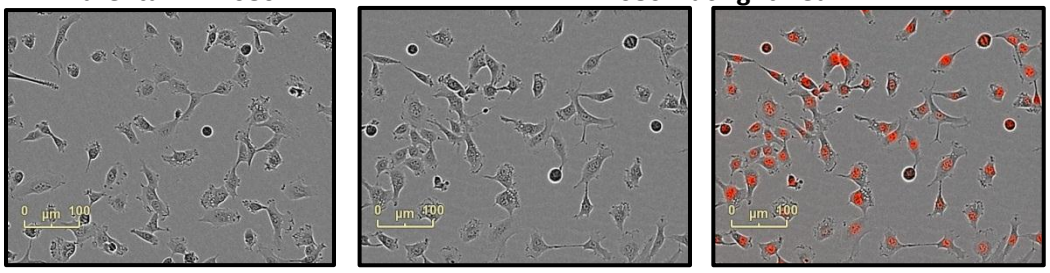
(Optional) Hexadimethrine Bromide; aka Polybrene® (2 mg/mL stock solution; Sigma-Aldrich: H9268)

Validation Assays (Quality Control Testing)

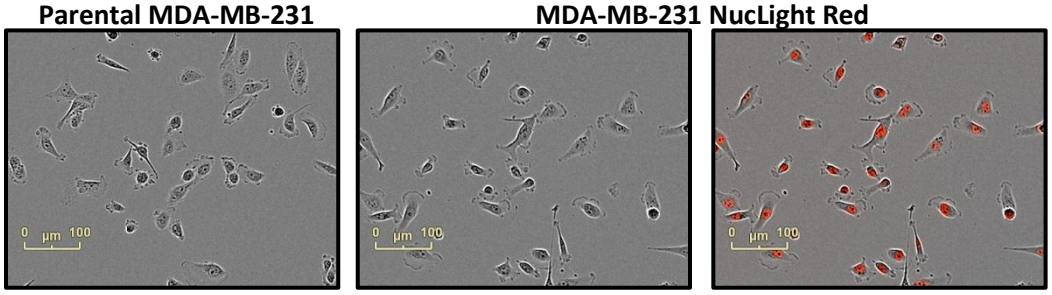
The following experiments were completed using an IncuCyte ZOOM™ (10x)
NOTE: All validation assays and images were collected using the puromycin resistance marker

1. Morphological Comparison – No morphological differences between transduced and parental populations.

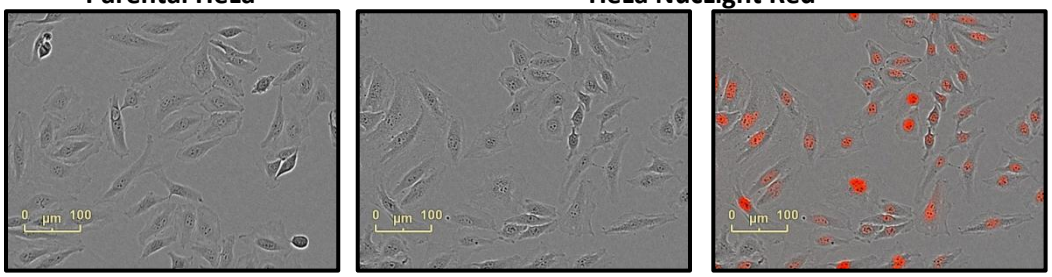
Parental HT-1080 **HT-1080 NucLight Red**



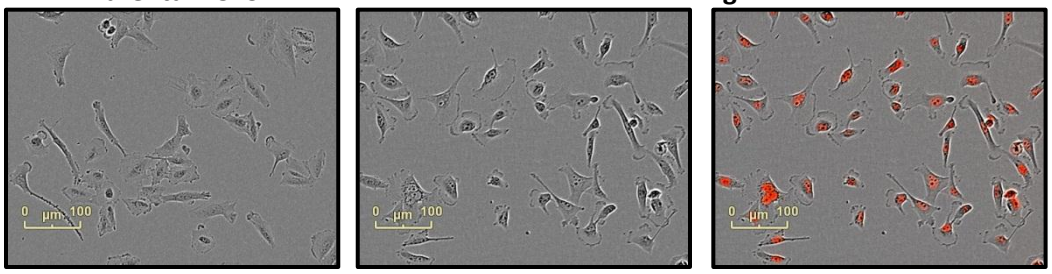
Parental MDA-MB-231 **MDA-MB-231 NucLight Red**



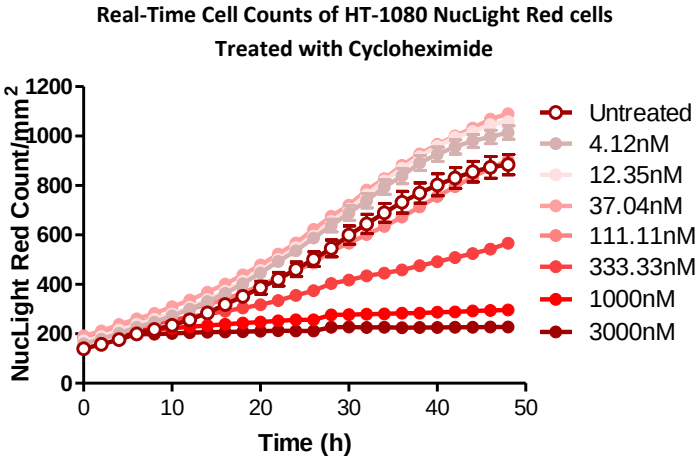
Parental HeLa **HeLa NucLight Red**

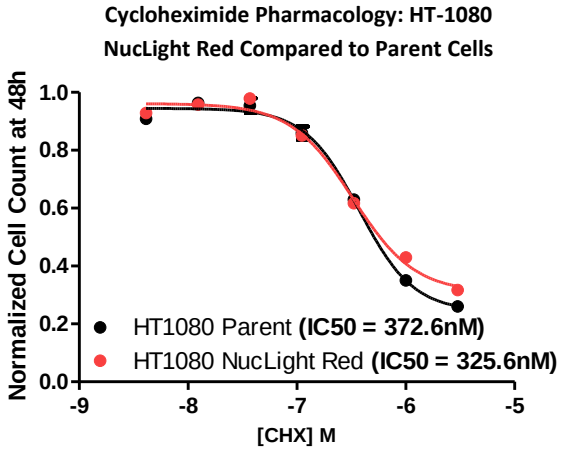


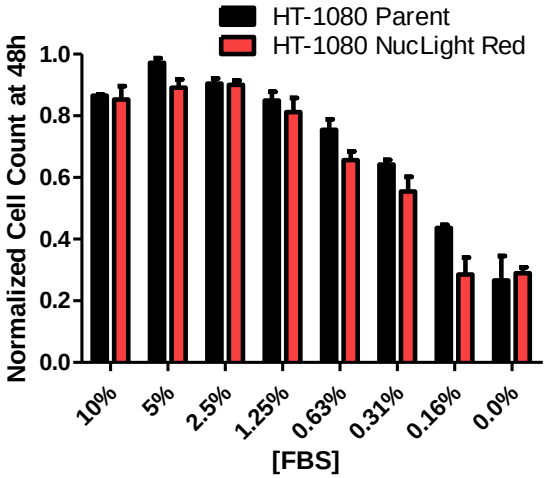
Parental A549 **A549 NucLight Red**



2. Proliferation







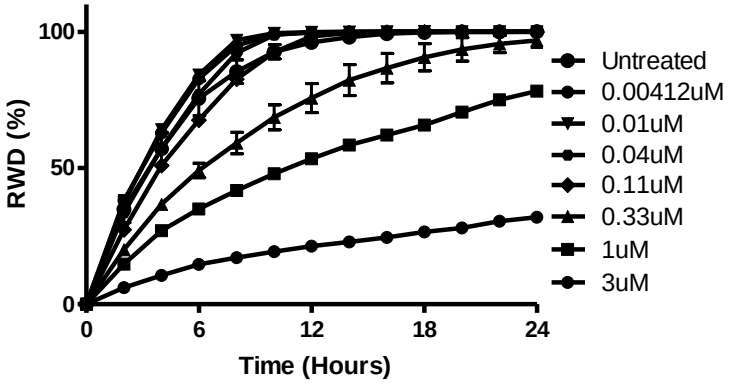
	Proliferation (CHX IC50)	
Cell Type	**Parent**	**NucLight Red**
HT-1080	372.6 nM	325.6 nM
HeLa	560.4 nM	979.1 nM
MDA-MB-231	335.8 nM	602.2 nM
A549	181.6 nM	246 nM

*Raw data can be found in individual Data Sheets for each cell line

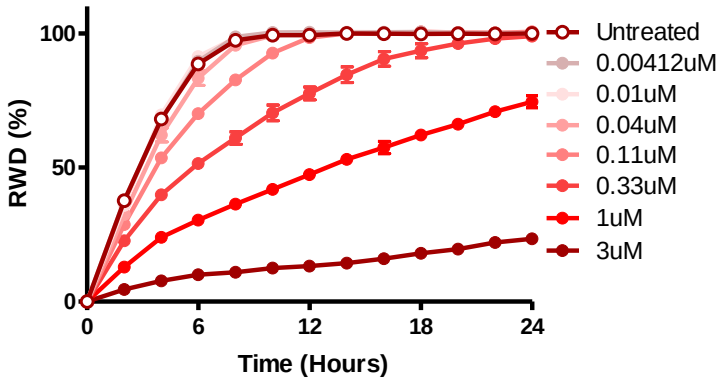
Results: Each of the cell lines listed above (HT-1080, MDA-MB-231, HeLa, and A549) have been extensively analyzed to determine if infection of Lentivirus or expression of NucLight Red nuclear label has a detrimental effect on cell proliferation. The kinetic graph (Top Left) illustrates the concentration response of HT-1080 NucLight Red cells to cycloheximide treatment. At the 48 hour endpoint, identically treated parental controls were stained with Vybrant DyeCycle Green and counted. Pharmacological analysis at the endpoint revealed similar cycloheximide IC50 concentrations for both parent and NucLight populations (Top Right). This analysis did not reveal a substantial shift in pharmacology in stable NucLight Red populations compared to identically treated parental cells (Table). Each cell type was also grown in reduced serum conditions (Bottom Left). Again, no differences in growth characteristics were observed between parent populations and stable populations expressing NucLight Red (raw data can be found in Product Data Sheets for each cell type). Together these data indicate that Lentivirus transduction and NucLight Red expression do not alter proliferation of cells relative to the parental controls.

3. Cell Migration

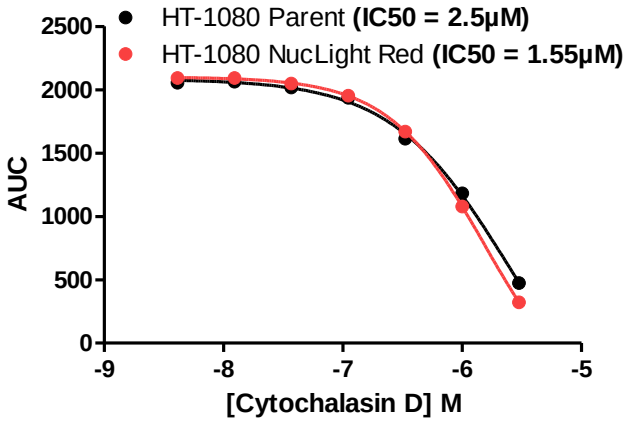
Kinetic Pharmacology: Cytochalasin D Treated Parental HT-1080 Cells



Kinetic Pharmacology: Cytochalasin D treated HT-1080 NucLight Red cells



Kinetic Pharmacology: AUC Analysis and IC50 Calculation



	Migration (CytoD IC50)	
Cell Type	**Parent**	**NucLight Red**
HT-1080	2.5 µM	1.55 µM
HeLa	640 nM	229 nM
MDA-MB-231	81.9 nM	84.7 nM
A549	100.7 nM	93.7 nM

*Raw data can be found in individual Data Sheets for each cell line

Results: The migration kinetics of each cell type was also analyzed. As an example, parental HT-1080s and the stable HT-1080 NucLight Red population were evaluated using the label-free Essen CellPlayer 96-well Cell Migration assay in conjunction with the Essen WoundMaker tool (Cat# 4443). Cells were treated with decreasing concentrations of cytochalasin D, a potent inhibitor of actin polymerization. Concentration dependent inhibition of wound closure, analyzed using Essen's Relative Wound Density (RWD) metric, was observed in both parental and stable HT-1080 NucLight Red cells at concentrations of CytoD ≥0.33 µM. Pharmacological analysis using the area under the curve (AUC) of the kinetic traces revealed similar IC50 values for cytochalasin D treatment. Summary statistics for each cell type can be found in the associated Table.

Protocols and Procedures

General Infection Protocol

1. Seed cells in growth media of choice at a density such that they are 15-35% confluent at time of infection. Incubate 24 hours, or enough time for cells to attach to plating surface.
   Example: Seed 10,000 HT-1080 cells in one well of a 24-well plate

2. Add Lentivirus at desired multiplicity of infection (MOI = TU/cell). An MOI of 3 is recommended for most cell types. However, an optimized MOI should be determined for each cell type in use, especially for transient assays. Polybrene® (1-8 µg/mL) can be used to enhance transduction of many cell types (Note: Some cell types are sensitive to Polybrene® (e.g. neurons).
   Example: 10,000 × 3 TU/cell (MOI) = 30,000 TU; 30,000 TU ÷ 1.57×10^{6} TU/mL = 0.0191 mL or 19.1 µl. Transduction of HT-1080 cells can be greatly enhanced if transduced in the presence of Polybrene®. Recommended Polybrene concentrations range from 1-8 µg/mL depending on the cell type. For HT-1080 cells, 8 µg/mL Polybrene® is recommended.

3. Incubate at 37°C, 5% CO_2 for 24 hours.

4. Remove media and replace with fresh growth media.
   NOTE: Media should be treated as biohazardous waste and treated with a 10% bleach solution prior to disposal per waste disposal guidelines.

5. Return to incubator for an additional 24-48 hours, monitoring expression using an IncuCyte ZOOM™ or fluorescence microscope.

6. Pick up cells and distribute at desired density for experiment. For stable selection, proceed to step 7.

7. (Optional) Remove media and replace with fresh growth media containing Zeocin selection.
   Example: For HT-1080, A549, HeLa, and MDA-MB-231 cells, media containing 200 µg/mL Zeocin is sufficient for efficient killing of non-transduced cells.

8. Incubate for 72-96 hours, replacing media every 48 hours.

9. Maintain stable population in a maintenance concentration of selection media.
   Example: HT-1080, A549, HeLa, and MDA-MB-231 NucLight Red cells can be maintained in complete media containing 40-100 µg/mL Zeocin.

Optimizing Polybrene® Concentration

Optimal Polybrene® concentrations will vary depending on cell type. The following table provides transduction conditions for several common cell lines from both Essen's experience and reported from other sources. Please note, Polybrene® can be toxic to certain cell types (e.g. primary neurons). The standard Essen CellPlayer Cytotoxicity protocol can be used to evaluate the toxic effect of Polybrene® on your cells.

1. Plate cells at a range of cell densities and culture overnight.

2. Replace the culture medium with fresh medium containing a range of Polybrene® concentrations (0-8 µg/mL) in the presence of YOYO-1 (optional, recommended concentration 100 nM) and return to incubator overnight.

3. Monitor cells for loss of membrane integrity (or other marker of cell death), and examine culture for cell viability. Identify the highest concentration of Polybrene® that does not cause toxicity.

Use this optimized concentration of Polybrene® for subsequent optimization steps.

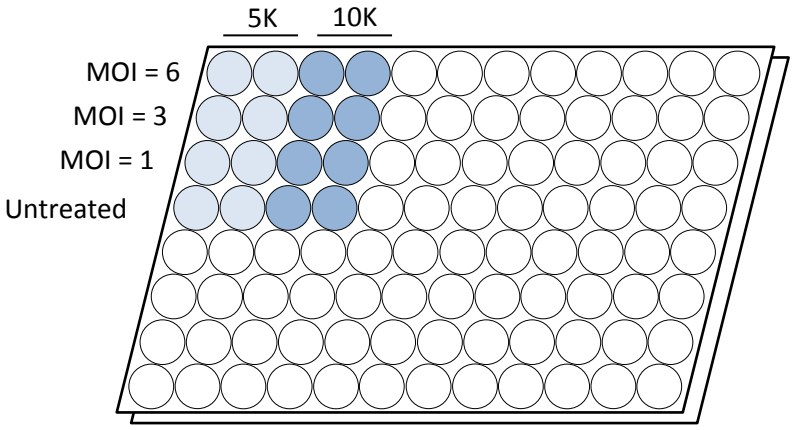
A549	Human lung carcinoma	3	8 µg/mL
Dermal Fibroblasts	Human primary dermal fibroblast	3	5 µg/mL
ECFC	Human endothelial colony forming cell	6	None
HEK293	Human embryonic kidney	3	8 µg/mL
HeLa	Human epithelial carcinoma	3	8 µg/mL
HT-1080	Human fibrosarcoma	3	8 µg/mL
HUVEC	Human primary umbilical vein endothelial	6	None
MCF10a	Human mammary fibrocystic disease	3	3-8 µg/mL
MCF7	Human mammary adenocarcinoma	3	3-8 µg/mL
MDA-MB-231	Human breast, adenocarcinoma	3	8 µg/mL
NIH-3T3	Mouse embryo fibroblast	6	8 µg/mL
SH-SY5Y	Human brain neuroblastoma	3	4 µg/mL

Optimizing Multiplicity of Infection (MOI)

Determining the optimal MOI for your cell line can be completed empirically in a 96-well plate.

1. Plate at least two densities of cells in a 96-well plate in appropriate medium.

   NOTE: Passage number can have a significant effect on lentiviral transduction efficiency. Low passage cells should be used in all experiments



2. Incubate cells overnight in a 37°C, 5% CO_2 incubator

3. Prepare transduction media plus appropriate concentration of Polybrene® and replace growth media with transduction media.

4. After 24 hours, replace transduction media with growth media and return cells to incubator.

5. 48-72 hours after transduction, evaluate cells for efficiency of transduction:

   Example: If using NucLight Red Lenti, stain cells with Vybrant DyeCycle Green following our standard no-wash protocol (Final concentration of 1 µM). Using the IncuCyte ZOOM™, count the number of objects prior to staining, then count the number of objects post stain. From these values, calculate transduction efficiency for each MOI. If using CytoLight Red Lenti, quantifying accurate transduction efficiencies can be a challenge as cells can be difficult to accurately mask. In this case, a qualitative measure may be more appropriate. If accurate transduction efficiencies are required, a flow cytometry approach may be more appropriate.

Optimizing Antibiotic Selection:

If the generation of stable populations or clones is the primary objective, optimization of MOI and transduction conditions is less important as the selection process well eliminate non- or low-expressing cells within the population. However, a kill curve with several concentrations of Zeocin should be completed on non-transduced cells to determine the lowest concentration of selection that is required to efficiently eliminate non-transduced cells.

Safety Considerations

The backbone of the Lentivirus particles in this system has been modified to improve their safety and minimize their relation to the wild-type, human HIV-1 virus. These modifications include:

- The lentiviral particles are replication-incompetent and only carry the non-oncogenic gene of interest.
- A deletion in the 3' LTR (ΔU3) resulting in "self-inactivation" (SIN) of the Lentivirus after transduction and genomic integration of the target cell (Yee et al., 1987; Yu et al., 1986; Zufferey *et al.*, 1998). This alteration renders the lentiviral genome incapable of producing packageable virus following host integration.
- The envelope is psueudotyped with the VSV-G gene from Vesicular Stomatitis Virus place of the HIV-1 envelope (Burns et al., 1993; Emi et al., 1991; Yee et al., 1994).

Replication-defective lentiviral vectors, such as the 3rd generation vector provided in this product, are not known to cause any diseases in humans or animals. However, lentivirus particles still pose some biohazardous risk because they can transduce primary human cells and can integrate into the host cell genome thus posing some risk of insertional mutagenesis. For this reason, **we highly recommend that you treat lentiviral stocks as Biosafety Level 2 (BSL-2, BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.**

For more information about the BL-2 guidelines and Lentivirus handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm. You may also refer to the NIH's Lentivirus containment guidelines at:
http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

Institutional Guidelines: Safety requirements for use and handling of lentiviruses may vary at individual institutions. We recommend consulting your institution's health and safety guidelines and/or officers prior to implementing the use of these reagents in your experiments.

A detailed discussion of lentiviral vectors is provided in Pauwels, K. et al (2009). State-of-the-art lentiviral vectors for research use: Risk assessment and biosafety recommendations. *Curr. Gene Ther.* 9: 459-474.

Related Products

NucLight/CytoLight Reagents:

Cat.# 4475 CellPlayer NucLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4476 CellPlayer NucLight Red (Lenti, EF-1 alpha, puro)
Cat.# 4481 CellPlayer CytoLight Green (Lenti, EF-1 alpha, puro)
Cat.# 4482 CellPlayer CytoLight Red (Lenti, EF-1 alpha, puro)
Cat.# 4513 CellPlayer CytoLight Green (Lenti, CMV, no selection)

NucLight Cell Lines:

Cat.# 4485 CellPlayer HT-1080 NucLight Red
Cat.# 4486 CellPlayer HT-1080 NucLight Green
Cat.# 4487 CellPlayer MDA-MB-231 NucLight Red
Cat.# 4488 CellPlayer MDA-MB-231 NucLight Green
Cat.# 4489 CellPlayer HeLa NucLight Red
Cat.# 4490 CellPlayer HeLa NucLight Green
Cat.# 4491 CellPlayer A549 NucLight Red
Cat.# 4492 CellPlayer A549 NucLight Green
Cat.# 4506 CellPlayer HUVEC NucLight Green
Cat.# 4511 CellPlayer Neuro-2a NucLight Green
Cat.# 4453 CellPlayer HUVEC CytoLight Green
Cat.# 4512 CellPlayer Neuro-2a NucLight Red

For additional information on this and other products, please contact Essen BioScience at: sales@essenbio.com.

For research use only. Not for therapeutic or diagnostic use.

Licenses and Warranty

Essen BioScience warrants that this product performs as described on the product label and in all literature associated with the sale of said product when used in accordance with the described protocol. This limited warranty is the sole warranty. No other warranties exist that extend beyond this warranty, either expressed or implied. Essen BioScience disclaims any implied warranty of merchantability or warranty of fitness for a particular purpose. Essen BioScience disclaims any responsibility for injury or damage and shall not be liable for any proximate, incidental or consequential damages in connection with this product.

If it is proven to the satisfaction of Essen BioScience that this product fails to meet performance specifications, Essen BioScience's sole obligation, at the option of Essen BioScience, is to replace the product or provide the purchaser with credit at or below the original purchase price. This limited warranty does not extend to anyone other than the purchaser. Notice of suboptimal performance must be made to Essen BioScience within 30 days of receipt of the product.

This Essen BioScience product contain proprietary nucleic acid(s) coding for proprietary fluorescent protein(s) being, including its derivatives or modifications, the subject of pending patent applications and/or patents owned by Evrogen JSC (hereinafter "Evrogen Fluorescent Proteins").

The purchase of Essen BioScience products incorporating these fluorescent proteins conveys to the buyer the non-transferable right to use Evrogen Fluorescent Proteins only for research conducted by the buyer. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product or to use Evrogen Fluorescent Proteins for commercial purposes. The right to use Evrogen Fluorescent Proteins specifically excludes the right to validate or screen compounds for commercial purposes. For information on commercial licensing, contact Evrogen Licensing Department, email: license@evrogen.com.