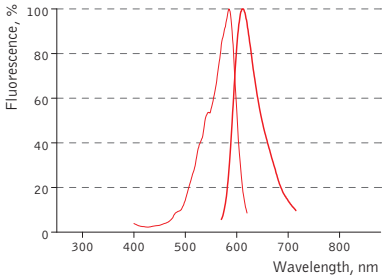
Genetically-encoded photoinducible cell cycle inhibitor ArrestRed

- Reversible inhibition of cell cycle progression
- Activation by green light irradiation
- Direct expression and easy visualization in cell nuclei
- No exogenous chemical compounds required
- Recommended for transient blockage of cell division *in vitro* and *in vivo*



ArrestRed normalized excitation (thin line) and emission (thick line) spectra.
Complete ArrestRed spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com

ArrestRed (the scientific name is H2B-tKR) is a novel optogenetic tool, which can be used to study the roles of specific cell populations in development, regeneration, and carcinogenesis. This is a chimeric protein composed of a tandem version of genetically-encoded photosensitizer KillerRed fused to histone H2B protein [Serebrovskaya et al. 2011]. Expression of ArrestRed in mammalian cells results in correct chromatin labeling and does not interfere with cellular division. The illumination of the ArrestRed expressing cells by intense green light leads to blockage of cell proliferation. The effect of ArrestRed activation lasts for about 24 hours, after that approximately 90% of ArrestRed expressing cells resume division. Repeated light illuminations allow to maintain cells in the non-dividing state for longer periods.
The inhibitory effect of ArrestRed on cell cycle progression is attributed to generation of reactive oxygen species (ROS) upon ArrestRed activation [Bulina et al. 2006]. Light-induced generation of ROS leads to massive damage of genomic DNA and activation of repair machinery. In turn, it causes cell cycle checkpoints activation and cell cycle arrest. After successful DNA reparation interphase cells restore normal proliferation.

Main properties of ArrestRed

Characteristic	
Molecular weight, kDa	70
Polypeptide length, aa	637
Aggregation	no
Maturation rate at 37°C	slow
Activating light	green (e.g. 540-580 nm)
Fluorescence color	red
Excitation maximum, nm	585
Emission maximum, nm	610
Quantum yield	0.25
Extinction coefficient, $M^{-1}cm^{-1}$	90 000
Brightness*	22.6

* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.

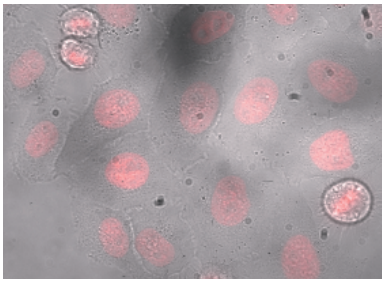
Performance and use

ArrestRed can be used for selective inhibition of cell cycle progression *in vitro* and *in vivo*. It can be expressed and activated in various experimental systems, including mammalian cells and *Xenopus* laevis.
Mammalian cells transiently transfected with ArrestRed expression vector give detectable red fluorescent signals in 24 hours after transfection.
Before light activation, ArrestRed enables correct chromatin labeling and does not interfere with cellular division.

Cell cycle distribution in asynchronously growing populations of parental HeLa Kyoto and stably transfected HeLa Kyoto ArrestRed cells.

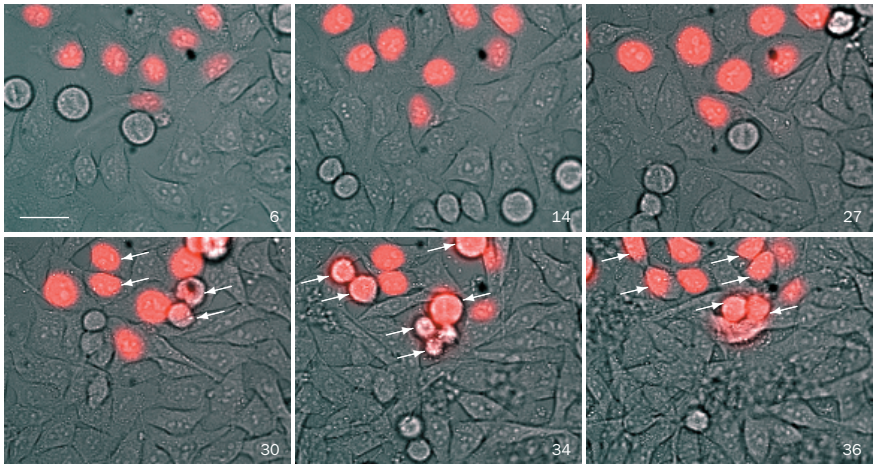
	HeLa Kyoto	HeLa Kyoto ArrestRed
G1	40%	35%
S	40%	45%
G2/M	20%	20%

Cells were analyzed by flow cytometry after staining with propidium iodide for the DNA content.



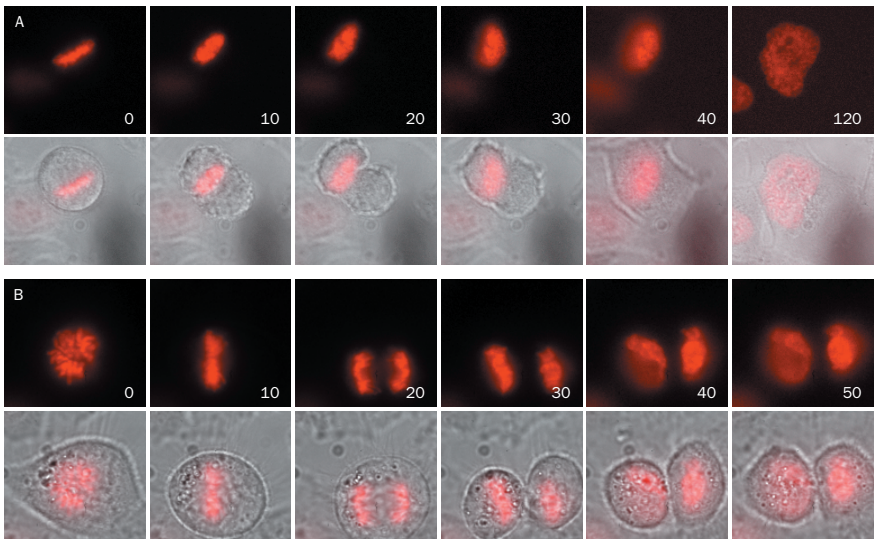
Visualization of ArrestRed in stably transfected HeLa Kyoto cell line. Overlay of red fluorescence and transmitted light are shown. Leica AF6000 LX imaging system (light source: a 120W HXP short arc lamp; filter set: a standard TX2 filter set: excitation BP560/40, emission BP645/75) was used for detection of red fluorescence from ArrestRed.

Effects of light-activated ArrestRed on cell division *in vitro*: Activation of ArrestRed in either transiently or stably transfected HeLa cells results in complete blockage of cell division for about 24 hours. During this time, cell nuclei have interphase morphology, and no cells undergo division. At the same time, most cells remain viable with no membrane blebbing, loss of attachments, cell shrinkage, or other signs of cell death. Over a second 24-h period, (24-48 h after activation of ArrestRed) approximately 90% of the ArrestRed-transfected cells undergo mitosis.



Time-lapse images of representative HeLa cells after activation of ArrestRed by green light illumination. Overlay of red fluorescence and transmitted light are shown (numbers indicate time in hours). Note that in contrast to non-transfected cells, ArrestRed expressing cells do not divide for 27 h, and then undergo mitosis normally (arrows point mitotic or newly appeared daughter cells).

Activation of ArrestRed in mitotic cells results in nondisjunction of chromosomes and the cells are unable to complete division normally. In the end the cells return to interphase morphology with a decondensed tetraploid chromatin.

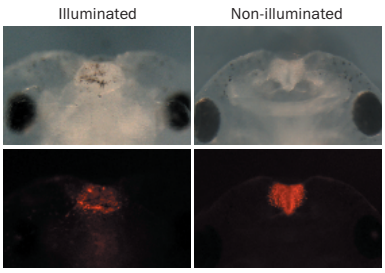


Time-lapse images of representative mitotic HeLa cells expressing ArrestRed. Red fluorescence and overlay of red fluorescence and transmitted light are shown (numbers indicate time in minutes). (A) A cell with ArrestRed activated during metaphase. (B) A control non-illuminated cell.

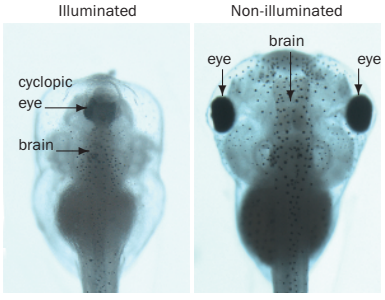
Effects of light-activated ArrestRed *in vivo*: The effects of light-induced activation of ArrestRed in the whole organism *in vivo* were demonstrated in transgenic *Xenopus* laevis embryos [Serebrovskaya et al. 2011].

In one set of experiments, the Xag2 promoter was used to specifically direct the ArrestRed expression in the cement gland, a provisory organ located at the rostral end of the embryonic head. Activation of ArrestRed (green light illumination with LED array, 525 nm, 45 mW/cm^2, 1 hour) in transgenic embryos at the early neurula stage leads to clear retardation of the cement gland differentiation observed at the tadpole stage.

In another set of experiments, the tissue-specific promoter of the homebox gene Xanf1 was used to specifically induce the ArrestRed expression in the cells of the anterior neural fold between the middle gastrula and the late neurula stages of the development. Activation of ArrestRed (green light illumination with LED array, 525 nm, 45 mW/cm^2, 1 hour) in transgenic embryos at the early-midneurula stages leads to various degrees of forebrain reduction accompanied by prominent optic stalk dysplasia, which in extreme cases resulted in a complete cyclopic phenotype observed at the tadpole stage.



Transgenic tadpoles expressing ArrestRed under the control of the cement gland-specific Xag2 promoter. Top panels – transmitted light; bottom panels – red fluorescence. ArrestRed was activated (left panels) or non-activated (right panels) by green light illumination at the early neurula stage. Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).



Transgenic tadpoles expressing ArrestRed under the control of the forebrain-specific Xanf1 promoter. ArrestRed was activated (left panel) or non-activated (right panel) by green light illumination at the early-midneurula stages. Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).

Recommended antibodies, filter sets, and activating parameters

ArrestRed can be recognized using Anti-KillerRed antibody (Cat.# AB961) available from Evrogen.
Before light activation, ArrestRed can be detected in cell nuclei using TRITC filter set or similar. Recommended Omega Optical filter sets are QMAX-Red and XF174.
ArrestRed is activated by green-light irradiation at 540-580 nm. Light activation of ArrestRed is accompanied by profound photobleaching (reduction of red fluorescent signal).
For activation of ArrestRed in cell cultures *in vitro* the Arc-lamp irradiation (540-580 nm, 0.5 W/cm^2, 2 min) is strongly recommended; laser-light irradiation in confocal mode is less efficient.
For activation of ArrestRed in the whole organism *in vivo* the Arc-lamp (illumination through 10x objective, 540-580 nm, 170 mW/cm^2, 20 min) or the LED array (525 nm, 45 mW/cm^2, 1 hour illumination) can be applied.
The source of irradiation, irradiation time and intensity of green light must be individually determined for particular biological system and instrumentation.
Excessive activation of ArrestRed may result in cell death.

Available variants and fusions

ArrestRed mammalian expression vectors contain ArrestRed coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas et al. 1996]. Humanized ArrestRed can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.
The available vectors encoding ArrestRed variants and fusions are listed below in the section ArrestRed-related products. For most updated product information, please visit Evrogen website www.evrogen.com.
If you need ArrestRed codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.

Licensing opportunities

Evrogen technology embodied in ArrestRed is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen at license@evrogen.com.

References

Bulina, M.E. et al. (2006). Nat Biotechnol, 24 (1): 95–99 / pmid: 16369538

Haas, J. et al. (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Serebrovskaya, E.O. et al. (2011). Biochem J, 435 (1): 65–71 / pmid: 21214518

ArrestRed-related products

Product	Cat.#	Description	Size
ArrestRed expression/source vectors			
pArrestRed	FP980	Mammalian expression vector encoding photoinducible cell cycle inhibitor ArrestRed	20 μg
Antibodies against ArrestRed			
Anti-KillerRed	AB961	Rabbit polyclonal antibody against KillerRed, ArrestRed, and JRed	100 μg

Please contact your local distributor for exact prices and delivery information.