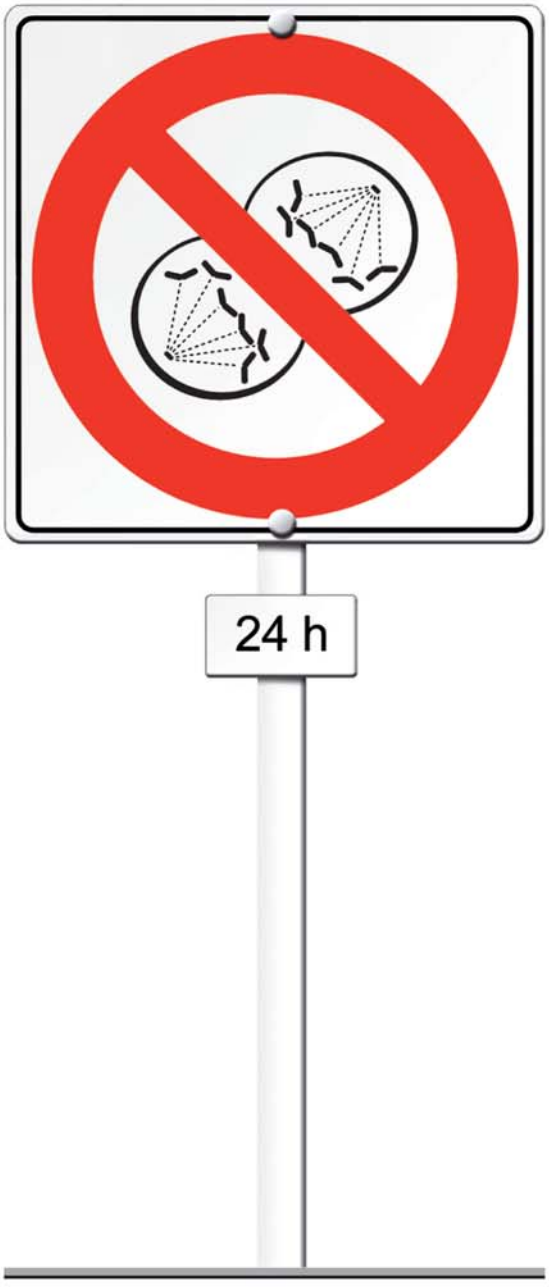


Photoinducible cell cycle inhibitor ArrestRed

Reversible inhibition of cell cycle progression
Activation by green light irradiation
Easy visualization in cell nuclei
No exogenous chemical compounds required

Genetically-encoded photoinducible cell cycle inhibitor ArrestRed

ArrestRed is a modified red fluorescent protein that can be easily expressed in different systems. The illumination of the ArrestRed expressing cells by green light leads to blockage of cell proliferation 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 ability to transiently block cell cycle progression makes ArrestRed a powerful optogenetic tool to study the roles of specific cell populations in development, regeneration, and carcinogenesis.

Performance and use

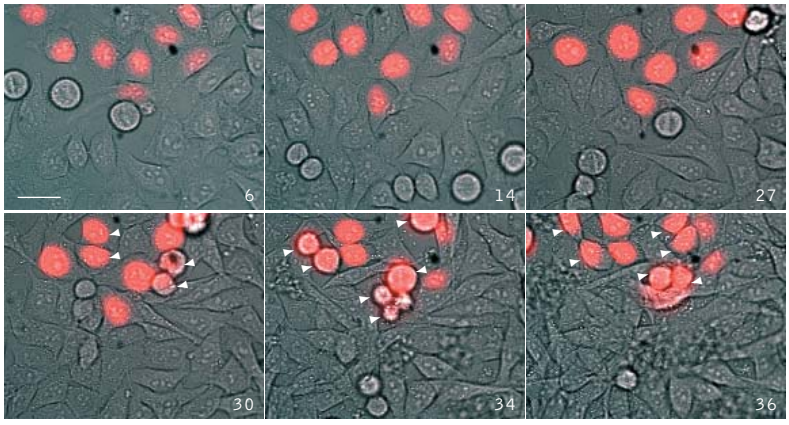
ArrestRed can be used for selective inhibition of cell cycle progression in various experimental systems. Before light activation, ArrestRed enables correct chromatin labeling and does not interfere with cellular division.

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.

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 h. 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.



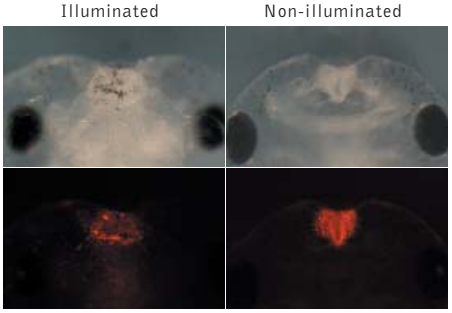
Time-lapse images of representative HeLa cells after activation of ArrestRed by green light illumination. Overlay of red fluorescence and transmitted light are shown (numbers indicate time in hours). Note that in contrast to non-transfected cells, ArrestRed expressing cells do not divide for 27 h, and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

ArrestRed-related products

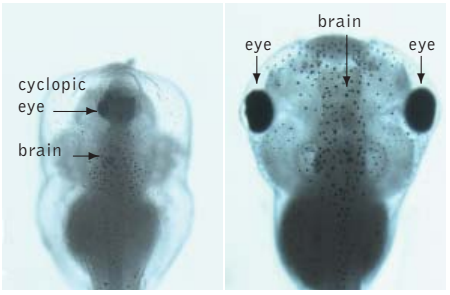
Product	Cat.#
pArrestRed expression/source vector	FP980

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

A The cement gland-specific Xag2 promoter



B The forebrain-specific Xanf1 promoter



Transgenic tadpoles expressing ArrestRed under the control of certain brain-specific promoters.
(A) ArrestRed was activated (left panels) or non-activated (right panels) at the early neurula stage (top panels – transmitted light, bottom panels – red fluorescence). (B) The left panel shows cyclopic tadpole developed after the ArrestRed activation at the early-midneurula stages, the right panel shows normal embryo developed in the dark. *Data courtesy of Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).*

REFERENCES

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

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