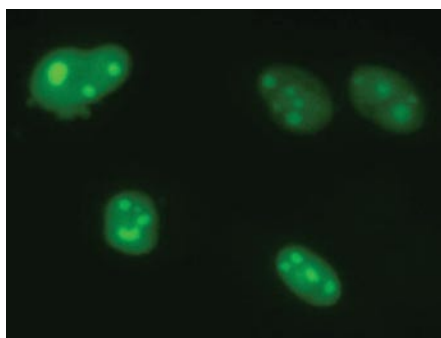


# Living Colors® pIRES2-AcGFP1-Nuc Vector

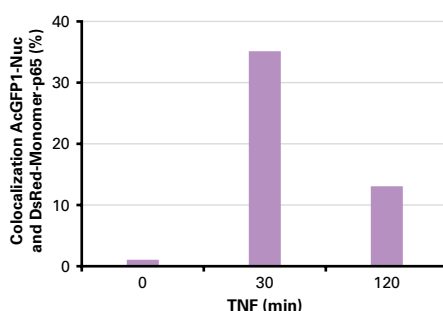
Identify transfected cells via distinct nuclear fluorescent labeling

- Easily detect transfected cells
- No nuclear dye staining necessary
- Ideal for High Content Screening (HCS) due to easy image quantification

The new **Living Colors pIRES2-AcGFP1-Nuc Vector** is designed for rapid identification and quantification of mammalian cells expressing your gene of interest. Nuclear staining is the most common method used by imaging software to quantify the number of cells in a microscopic field. Cells are normally stained using a dye such as DAPI, especially in High Content Screening (HCS) applications. While DAPI stains all cell nuclei uniformly, pIRES2-AcGFP1-Nuc allows selective fluorescent staining, facilitating detection and counting of transfected cells without an additional dye staining step. Only nuclei of cells expressing your gene of interest will be fluorescently labeled with AcGFP1-Nuc. Thus only cells expressing your gene of interest will be counted and analyzed (Figure 1).



**Figure 1.** HeLa cells were transiently transfected with pIRES2-AcGFP1-Nuc Vector using a lipid-based transfection method. 24 hr after transfection, cells were fixed in 3% paraformaldehyde and visualized using a Chroma filter set (Ex.:460/40; Em.: 515/30) on a Zeiss Axioskop fluorescence microscope. Images of cells (40X) expressing AcGFP1-Nuc are shown.



**Figure 2.** Use of pIRES2-AcGFP1-Nuc in a nuclear translocation assay. HeLa cells were transfected with p65(NFκB)-DsRed-Monomer-IRES2-AcGFP1-Nuc and pCMV-lkB (ratio 7:1). 24 hours after transfection, cells were treated with TNF-α (100 ng/ml) for either 30 min or 2 hr. Cells were fixed and visualized using a Zeiss Axioskop fluorescence microscope (AcGFP1: Ex.:460/40; Em.: 515/30; DsRed-Monomer Ex.: 540/40; Em.: 600/50). Colocalization of green and red fluorescence in the nucleus with or without TNF-α was quantified and shown as % colocalization.

The selective staining of nuclei is accomplished by the presence of an IRES (Internal Ribosome Entry Site) sequence (1, 2) between your gene of interest and the sequence encoding the green fluorescent protein AcGFP1-Nuc.

Three identical copies of the nuclear localization signal (NLS) of simian virus 40 T-antigen are fused in tandem to the C-terminus of AcGFP1 to target it to the nucleus (3–5). Since your gene of interest and AcGFP1-Nuc are translated from the same mRNA transcript via the IRES, the intensity of fluorescence of AcGFP1-Nuc is directly proportional to the expression level of your gene of interest. There is no risk of altering the biological activity of the protein since your gene of interest is not expressed as a fusion with AcGFP1-Nuc.

## Monitoring nuclear transport

Although many proteins such as transcription factors are localized in the cytosol, they translocate to the nucleus in response

Product	Size	Cat. No.
pIRES2-AcGFP1-Nuc Vector	20 µg	632515

## Related Products

- pIRES2-AcGFP1 Vector (Cat. No. 632435)
- pIRES2-ZsGreen1 Vector (Cat. No. 632478)
- pIRES2-DsRed2 Vector (Cat. No. 632420)
- pIRES2-DsRed-Express Vector (Cat. No. 632463)
- pDsRed2-Nuc Vector (Cat. No. 632408)
- pHcRed1-Mito Vector (Cat. No. 632434)

## Notice to Purchaser

Please see the CMV Sequence, DsRed-Express, IRES-Containing Vectors, and Living Colors® legal statements on page 33.

to an external stimulus. Potential translocations in or out of the nucleus can be easily monitored by cloning your gene of interest fused to another fluorescent protein such as DsRed-Monomer, upstream of pIRES2-AcGFP1-Nuc.

Transfecting cells with such constructs enables easy detection of bright green fluorescence from nuclei of transfected cells. Upon stimulation, translocation of the DsRed-tagged protein from the cytosol to the nucleus can be monitored by detecting colocalized green and red fluorescence in the nucleus. The ease of quantifying transient translocation of p65 in transfected HeLa cells from the cytosol to the nucleus is demonstrated in Figure 2. The Living Colors pIRES-AcGFP1-Nuc Vector allows easy detection of transfected cells without nuclear dye staining, making it an ideal choice for High Content Screening applications.

## References

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4. Lanford, R. E., et al. (1986) *Cell* **46**(4):575–582.
5. Fischer-Fantuzzi, L. & Vesco, C. (1988) *Mol. Cell. Biol.* **8**(12):5495–5503.