Achieve the Protein Expression Level You Need with the Mammalian HaloTag® 7 Flexi® Vectors

ABSTRACT
A series of vectors containing deletions of human cytomegalovirus (CMV) immediate-early enhancer/promoter were created to provide different constitutive expression levels of HaloTag® 7 fusion proteins, providing greater control over the level of protein expression. This allows expression of enough fusion protein to image or capture but not so much to disturb normal function of the protein partner fused to the HaloTag® 7 protein.

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Sample Pack (Cat.# G3780)
The HaloTag® 7 CMV promoter deletion series and the full-length CMV promoter are available as a sample pack. This pack allows the cloning of a protein coding region into a donor vector and then transfer of the protein coding region to any or all of the complete set of HaloTag® 7 vectors.

INTRODUCTION
For protein overproduction with the purpose of over-expressing proteins, the human cytomegalovirus (CMV) immediate-early enhancer/promoter is often chosen for its robust expression levels. However, this high level of production can potentially disrupt the normal behavior of the protein or the physiology of the cell and may need to be adjusted. While protein overexpression studies yield important clues to the normal functioning of a gene product in the cell, the results must be interpreted with caution. When expressing protein fused to a tag such as the improved HaloTag® 7 reporter protein (1), these same concerns apply.

The HaloTag® 7 protein is an engineered, catalytically inactive derivative of a hydrolase that forms a covalent bond with HaloTag® Ligands. HaloTag® protein fusion can be used either for capture of the fusion and its molecular partners in the cell or for labeling with a variety of fluoros. We developed the HaloTag® 7 Flexi® Vector CMV deletion series (a–d), a series of human CMV promoter deletions that offer different constitutive expression levels for HaloTag® fusion proteins. This allows researchers to express just enough protein for their application.

DIFFERENT EXPRESSION LEVELS: HALOTAG® 7 FLEXI® VECTOR CMV DELETION SERIES
A series of 5' deletions of the human CMV promoter were synthesized and incorporated into a Flexi® Vector format such that either firefly or Renilla luciferase-HaloTag® 7 fusion proteins were expressed. The resulting activity levels were screened to create a series of successive deletions that provided a five- to tenfold drop in expression from one deletion to another. Deletions to –116 (CMVd1), –67 (CMVd2) and –59 (CMVd3) were chosen to make up the series (Figure 1). Expression levels depend on the cell type and the protein being expressed, and with the exception of CHO cells, the CMV deletions provide a consistent drop in expression with each successive deletion (Figure 2). HaloTag® 7 activity mirrored luciferase activity (data not shown).

To complement the C-terminal HaloTag® 7 Vectors, the same promoters were used to build fusion Flexi® Vectors appending HaloTag® 7 on the amino-terminus of the protein of interest. Luc2, an engineered brighter version of firefly luciferase, was cloned into all vectors. Activity levels for luciferase (Figure 3) and HaloTag® 7 (data not shown) were similar, with HaloTag® 7 activity mirroring luciferase activity. Ampicillin- and kanamycin-resistant versions of each vector were made and provide similar expression levels. In addition, both C-terminal and N-terminal HaloTag® 7 versions of each promoter provide similar expression levels.
CMV DELETION SERIES SAMPLE PACK

The HaloTag® 7 CMV promoter deletion series and the full-length CMV promoter are available as a sample pack (Cat.# G3780). This pack allows the cloning of a protein coding region into a donor vector, pFN21A, and then transfer of the protein coding region to any or all of the complete set of mammalian HaloTag® 7 vectors (Figure 4).

P65/NFκB FUNCTIONAL ASSESSMENT AT DIFFERENT EXPRESSION LEVELS

The model system of p65/NFκB was used to examine proper function and cellular localization with HaloTag® 7 under different expression levels. In the absence of stimulation, the p65 protein resides primarily in the cytoplasm in most cells. Upon stimulation with the agonist TNF-α, p65 translocates to the nucleus, where it has been shown to bind directly and activate transcription of several targets, including the 1kB promoter. The HaloCHIP™
method, which was designed to study the intracellular binding of HaloTag® fusion proteins to DNA without the use of antibodies, was employed to study the DNA binding response to TNF-α of the various p65-HaloTag® fusion constructs. Analysis of the IkB promoter isolated from the HaloCHIP™ process (Figure 5) demonstrates that only CMVd2 shows the proper increased DNA binding response to the TNF-α stimulation. Given the sensitivity of the p65/NFκB system to absolute protein levels and regulatory feedback loops, this demonstrates an example where low levels of expression are better suited to study proper physiology.

p65-HaloTag® fusion protein cellular localization was difficult to determine when using the full-length CMV protein, as most of the cells were saturated with labeled fusion protein. However, the CMV deletion strains did provide appropriately labeled cells suitable for imaging with CMVd2, providing an optimal expression level. In cells expression p65 HaloTag® 7 fusion with the CMVd2 promoter, it is easy to observe cytoplasmic-nucleus migration during a time-course treatment with TNF-α (Figure 6). These results are consistent with those observed using the HaloCHIP™ method (5).

SUMMARY

Overexpression of proteins in a cellular environment can potentially perturb protein function. To address these concerns, the HaloTag® 7 CMV promoter deletion series was created. This series allows fine tuning of protein expression levels as well as easy shuffling of the gene of interest between vectors. Proteins can be expressed at this level to study proper physiology combined with an easy, sensitive means of detection and purification.

Figure 5. Agonist-dependent DNA binding of p65-HaloTag® 7 at varying expression levels using the HaloCHIP™ method. HeLa cells (4 × 10⁴) were transfected with various p65-HaloTag® 7 constructs (pFC14, pFC15, and pFC16), then treated without or with TNF-α for 30 minutes. Sample crosslinking and processing was performed as described in the HaloCHIP™ System Technical Manual, TM075. DNA fragments obtained from the HaloCHIP™ procedure were further purified, and the p65-specific promoter IkB was amplified using standard PCR (33 cycles). The signal-to-background ratios for each construct (+/- TNF-α) were determined and plotted.

Figure 6. Agonist-dependent translocation of p65-HaloTag® 7 protein. HeLa cells (4 × 10⁴) were plated in CG chamber (Nunc) and transiently transfected with the p65-HaloTag® 7 constructs using LT1 (Mirus). 24-hour-post-transfection cells were labeled with HaloTag® TMR Ligand as described in reference 3. Unbound ligand was washed out, and cells were imaged on an Olympus PV500 confocal microscope using the appropriate filter sets. For functional assay, cells were challenged with TNF-α (10 ng/ml), and images were collected every 10 minutes. p65-HaloTag® 7 moves into the nucleus within 10 minutes and moves back to the cytoplasm by 60 minutes.

REFERENCES

1. Encell, L. et al. (2008) (manuscript submitted)

ORDERING INFORMATION

Product

HaloTag® 7 Flexi® Vectors — CMV Deletion Series Sample Pack — 9 × 2 μg G3780

* All Vectors are supplied in 20 μg aliquots.
Patent Pending.

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