IRES Expression Clones

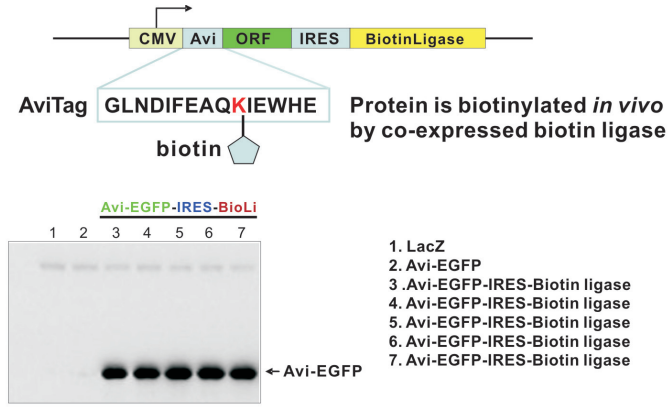

Figure 3. Specific biotinylation of AviTag-eGFP by *E. coli* biotin ligase in 293 cells. Biotin ligase and Avi-eGFP are expressed from the same CMV promoter via the IRES technology.

Promoter	Cell type	Fusion tag
CMV	Mammalian	C-3xHA+IRES-eGFP C-Flag+IRES-eGFP C-His+IRES-eGFP C-Myc+IRES-eGFP IRES2-eGFP N-Avi+IRES-Biotin ligase C-Avi+IRES-Biotin ligase
CMV (lenti vector)	difficult-to- transfect mammalian	IRES-eGFP IRES-eYFP IRES-eCFP IRES-mCherry IRES-luciferase C-Myc+IRES-eGFP C-Myc+IRES-eYFP C-Myc+IRES-eCFP C-Myc+IRES-mCherry C-Myc+IRES-luciferase C-3xHA+IRES-eGFP C-Flag+IRES-eGFP N-Avi+IRES-Biotin ligase C-Avi+IRES-Biotin ligase IRES-Neomycin C-Myc+IRES-Neomycin
PGK (lenti vector)	difficult-to- transfect mammalian	IRES-eGFP C-Myc+IRES-eGFP

To order

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GeneCopoeia™ Expressway to Discovery

HaloTag® Expression Clones

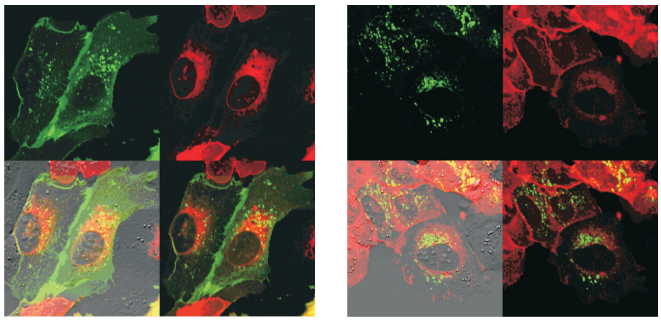
Overview

HaloTag® technology

The HaloTag is a multi-functional protein tag that binds covalently and specifically to a variety of synthetic HaloTag® ligands, which enables tagged proteins to be labeled with fluorophores for both *in vitro* and *in vivo* imaging or with affinity agents for purification.

OmicsLink™ HaloTag® ORF expression clones

Over 45,000 human and mouse ORF expression clones are available in mammalian and lentiviral vectors with either N- or C-terminal HaloTag®. They are expression-ready and fully sequence verified.



Time 0 Time 12 hours

Figure 1. Spatial and temporal separation of proteins using HaloTag® technology. Twelve hours after cell labeling, imaging showed the differentially labeled proteins as they moved from the cytoplasm to the membrane and as they internalized from the membrane. Detailed description of the experimental design and results can be found in Svendsen, S., et al., (Promega Notes 95 (2007) 16–19)

HaloTag® vector types

Promoter	Host cells	Tag	Protease site
CMV	Mammalian	N- or C-HaloTag®	Tev protease
CMV (lenti vector)	Difficult-to-transfect mammalian (primary, neurons, stem cells)	N- or C-HaloTag®	Tev protease
T7	Bacteria	N-HaloTag®	Tev protease

Advantages

Multi-functional tag

- In vivo and in vitro imaging
- Protein localization and co-localization
- Protein-DNA interaction
- Multiplex labeling for pulse-chase and translocation
- Protein enrichment and western blot analysis

Rapid and efficient detection

- Rapid and efficient coupling of synthetic reporter and affinity ligands

GeneCopoeia™
Expressway to Discovery

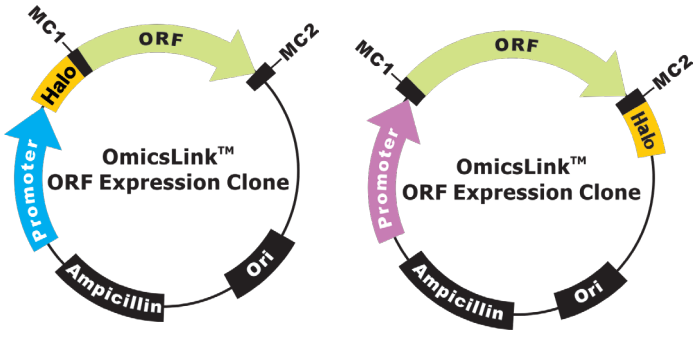


Figure 2. OmicsLink™ ORF cDNA expression clones with N- and C- HaloTag® in various expression vector systems.

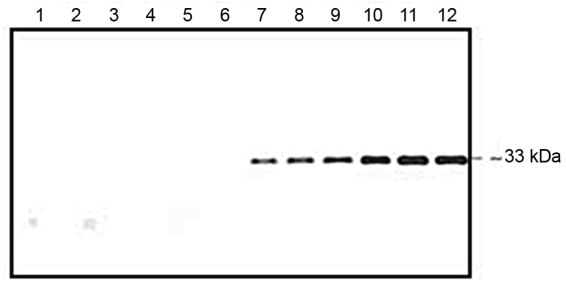


Figure 3. Fast, efficient and highly specific labeling of the HaloTag® proteins expressed in mammalian cells. CHO-K1 control cells (lanes 1–6) or cells transiently transfected with HaloTag® pHT2 expression clone (lanes 7–12) were labeled with 5 µM HaloTag® TMR Ligand for different periods of time at 37°C (0.5, 1, 2, 5, 15 and 30 minutes). Proteins were resolved by SDS-PAGE and analyzed on a Hitachi FMBIO® fluorescence scanner.

HaloTag® ligands and anti-HaloTag® antibody

Ligand	Excitation Maximum	Emission Maximum	Application
HaloTag® diAcFAM Ligand	494nm	526nm	Intracellular protein labeling
HaloTag® TMR Ligand	555nm	585nm	Intracellular protein labeling
HaloTag® CoumarinLigand	353nm	434nm	Intracellular protein labeling
Anti HaloTag® pAB	N/A	N/A	Labeling after fixing or protein enrichment

Please visit www.promega.com for more information on HaloTag® related products.

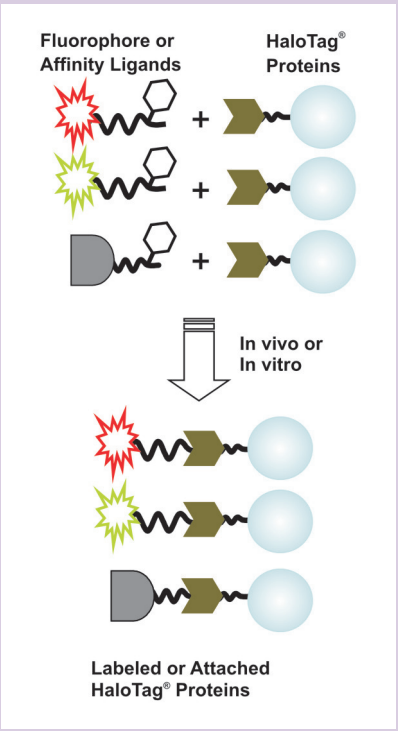


Figure 4. Diagram showing how HaloTag® works.

AviTag™ Expression Clones

Overview

AviTag™ technology

The AviTag system takes advantage of the strongest non-covalent interaction known in nature – that between biotin and avidin with a Kd = 10^{-15} M. The technology is based on the biotinylation of AviTag *in vitro* or *in vivo* and on the specific and reverse binding of avidin or streptavidin to biotin for immobilizing, purifying and visualizing proteins.

OmicsLink™ AviTag™ ORF expression clones

Over 45,000 expression-ready AviTagged human and mouse ORF clones are available in expression vectors with a choice of T7 or CMV promoters. They offer an easy solution for many applications, such as detection, isolation, imaging, localization and immoblization.

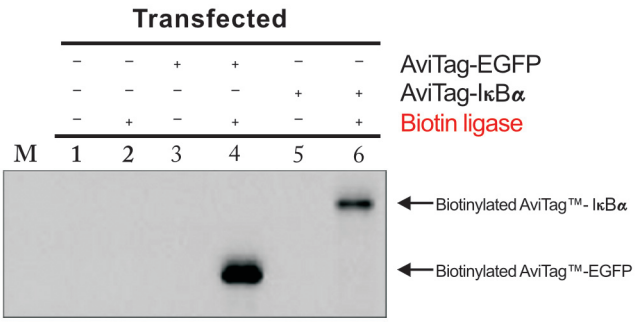


Figure 1. AviTag-eGFP or AviTag-IkBα expression plasmids were transfected into 293T cells alone or with co- transfected biotin ligase. Lysates were prepared 13 hours later. Extracts were prepared after transfection and resolved on a 12% SDS-PAGE gel. Biotinylated proteins were visualized on a western blot with streptavidin-HRP conjugate and chemiluminescent substrate.

Promoter	Host cells	Tag
CMV	Mammalian	N- or C-AviTag N- or C-Avi+IRES-Biotin ligase
CMV (lenti vector)	Difficult-to-transfect mammalian	N- or C-AviTag N- or C-Avi+IRES-Biotin ligase
T7	Cell free	N-AviSUMO N-HisAviSUMO N-HisSUMOAvi
T7	*E.Coli*	N-Avi

Advantages

Multi-functional tag

- Small-scale and high-throughput screening of protein-protein interactions
- Purification of AviTagged proteins using avidin
- Western blot, staining and sorting T cells with MHC-tetramers by visualizing AviTagged protein

Specific

- Biotinylation of the AviTag is highly specific. The chance for cross reaction is low when using biotinylation in protein purification

Sensitive

- Reproducible and reliable results for applications requiring high sensitivity, such as protein- protein interactions

AviTag™ Expression Clones

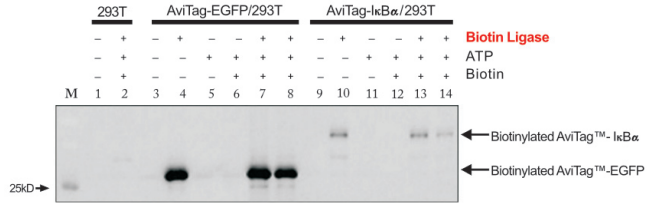


Figure 2. Extracts from 293T cells transfected with the AviTag-eGFP or AviTag-IkBα expression plasmid and untransfected controls were mixed with purified biotin ligase, biotin (50M) and ATP (10mM) as indicated in the figure. After incubation at 30°C (lanes 1-7, 9-13) or 4°C (lanes 8, 14) for 30 minutes, the mixtures were resolved by SDS-PAGE. Biotinylated proteins were visualized on a western blot with streptavidin-HRP conjugate and chemiluminescent substrate.

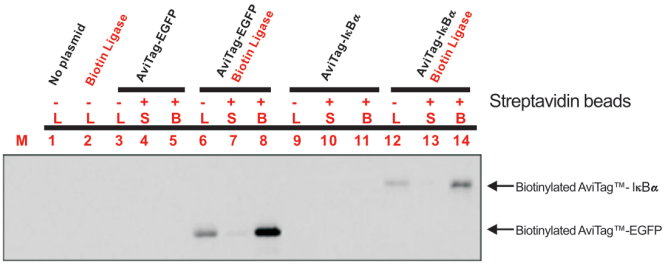


Figure 3. 293T cells were transfected with different combinations of AviTag expression plasmids and biotin ligase as indicated in the figure. Lysates were prepared 24 hours after transfection and incubated with streptavidin beads for 8 hours. Suspensions were centrifuged and pelleted beads were washed 3 times. Cell lysates (L), supernatants (S) and pelleted biotinylated protein bound to streptavidin beads (B) were resolved by SDS-PAGE. Biotinylated proteins were visualized on a western blot with streptavidin-HRP conjugate and chemiluminescent substrate.

Related products

Biotin protein ligase
Biotin solution
Positive controls
Anti-C-terminus AviTag antibody
Biotinylation strains

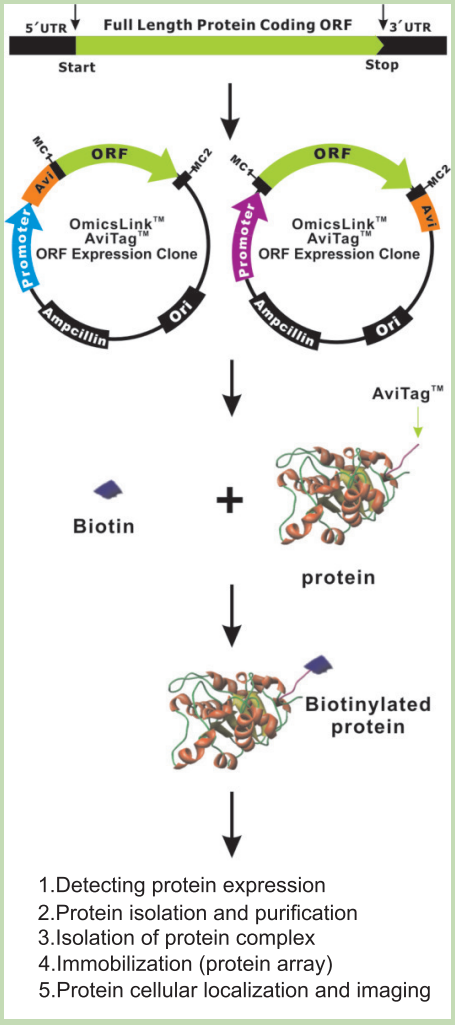


Figure 4. Diagram showing How AviTag works.

IRES Expression Clones

Overview

IRES technology

The Internal Ribosome Entry Site (IRES) technology allows coordinated and efficient co-expression of two genes using the same promoter in a single vector. Virtually any combination of genes is possible.

OmicsLink™ IRES ORF expression clones

More than 45,000 human and mouse ORF clones are now available in OmicsLink IRES expression vectors. These ORF expression clones contain various promoters, fusion tags, and other features which makes them suitable for expression studies and functional assays in a variety of transcription and translation systems.

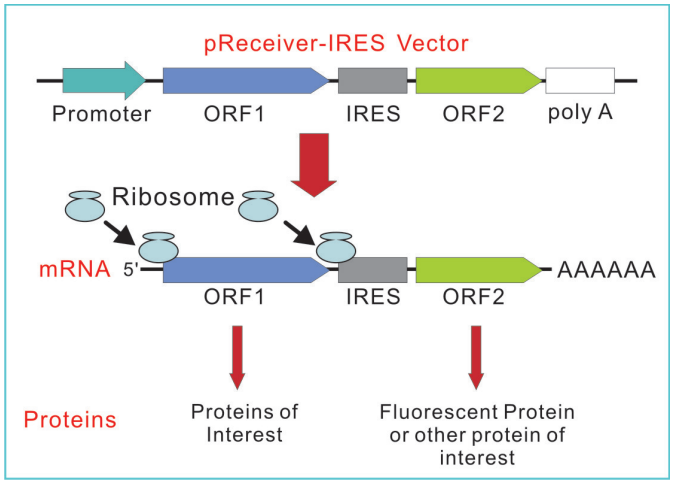


Figure 1. Diagram showing How IRES works.

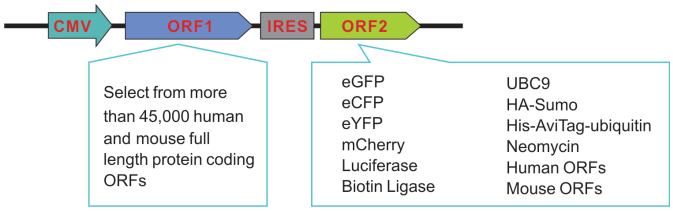


Figure 2. Vector choices for OmicsLink IRES ORF expression clones indicate wide selection of fusion tags, reporter genes, selection markers, etc. that can be co- expressed with the genes of interest.

Advantages

Multiple applications

- Monitor gene delivery efficiency by co-expression with reporter genes such as eGFP or luciferase
- Monitor protein modification by a specific modifier *in vivo* by co-expression with protein modifiers
- Allow *in vivo* biotinylation of AviTag™ fusion protein by co-expression with biotin ligase
- Allow more efficient selection and establishment of stably transfected cell lines by co-expression with selection marker genes

Minimal impact

- When reporter/assaying genes are co-expressed, the biological activities of the assaying proteins will be minimally affected

Eliminate co-transfection

- Eliminate concerns of co-transfection efficiency and other potential problems related to co-transfection of two expression plasmids