

Mapping Intracellular Protein:DNA Interactions: A more robust and efficient alternative to ChIP (Chromatin Immunoprecipitation).



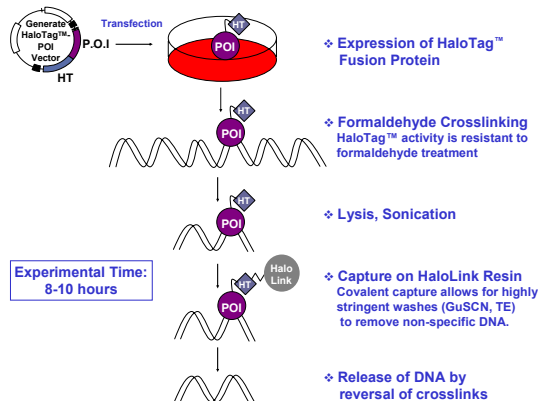
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Abstract and Introduction

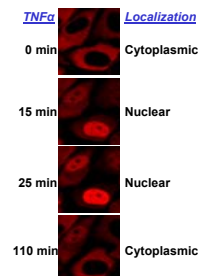
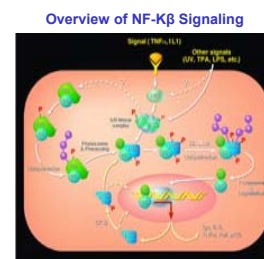
Regulation of chromatin structure and gene expression is essential for normal development and cellular growth. Transcriptional events are tightly controlled both spatially and temporally by specific protein:DNA interactions. While significant advances in DNA tiling and microarrays have allowed for genome-wide screening of chromatin recognition sites, current methods to isolate intracellular protein:DNA complexes remain cumbersome and require co-immunoprecipitation, a process inherently subject to capture of non-specific DNA and proteins. To address these concerns a novel method has been devised for the covalent capture of protein:DNA complexes which does not require the use of antibodies. Proteins of interest are expressed in cells as HaloTag™ fusion proteins, crosslinked to DNA, and then captured on HaloLink resin, which forms a highly specific, covalent interaction with HaloTag™. Due to the complete covalent linkage established between the resin and the crosslinked protein:DNA complexes, the resin can be stringently washed to remove non-specific DNA and proteins much more effectively than is possible by co-immunoprecipitation. The crosslinks are reversed to release purified DNA fragments from the resin. By improving specificity and reducing background interference during the isolation of protein:DNA complexes, this new methodology effectively increases the signal-to-noise ratio to permit detection of small changes in protein binding within a genome.

Covalent Capture of Chromatin Complexes Using HaloTag™ Technology



Functional p65-HaloTag™ in the NF-κB Signaling Pathway

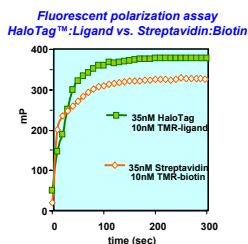
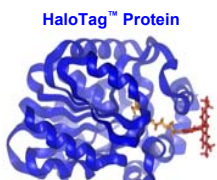
Imaging of p65-HT in living cells



HeLa cells transfected with p65-HT and treated with TNFα for various times. p65-HT is labeled with HaloTag™ TMR ligand.

Changes in cytoplasmic and nuclear localization of p65-HT treated with TNFα correspond to those seen for endogenous p65.

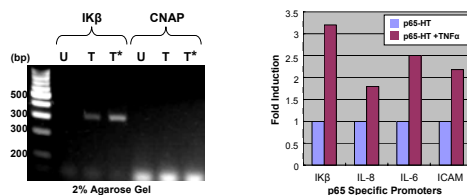
HaloTag™ Technology



- HaloTag™ (HT) is a monomeric protein.
- Covalently binds chloroalkane ligands with high specificity.
- Rapid binding kinetics comparable to streptavidin:biotin interaction

Specific Capture of p65 Promoters with p65-HaloTag™

Isolation and PCR amplification of the p65 Target Promoters from HeLa cells expressing p65-HT

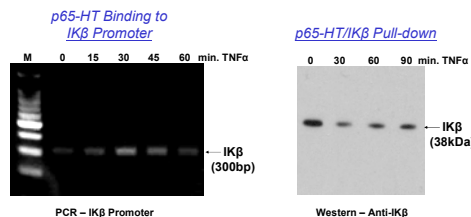


Untransfected (U), p65-HT transfected (T), or transfected and stimulated with TNFα (T*) HeLa cells were crosslinked and sonicated. Following the protocol above, lysates were incubated with HaloLink. Captured DNA fragments were released by reversal, purified, and amplified by PCR.

- Upon TNFα stimulation, increased binding by p65-HT to p65 specific promoters was observed, consistent with results using endogenous p65.
- Non-specific binding of p65-HT to a control promoter, CNAP1, is not observed.

Correlation of DNA and Protein Capture to Imaging Data

Isolation of Protein and DNA Complexes



HEK293 cells stably expressing p65-HT were treated with TNFα for various times. Using HaloLink resin, both protein:DNA and protein:protein complexes were isolated. Captured DNA fragments were amplified by PCR.

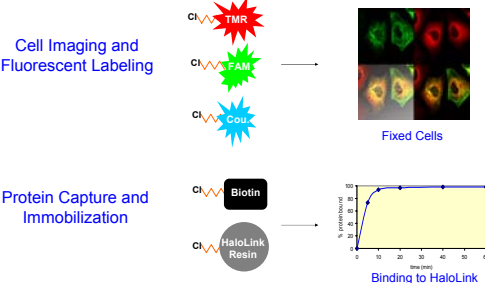
- IKβ promoter binding by p65-HT is inversely correlated to levels of cytoplasmic p65-HT/IKβ complexes.
- Time course results are in agreement with the model of the above NFKβ pathway and imaging data after TNFα stimulation.

The Versatility of HaloTag™

Applications

Ligands

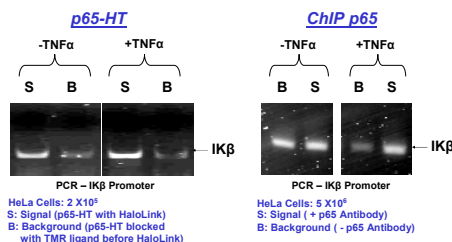
Examples



- Interchangeable HaloTag™ Ligands allow for multi-functional analysis of protein activity *in vivo* and *in vitro*.

Comparison with ChIP

Improved Assay Sensitivity with HaloTag™ Technology



- Both methods show a ~ 3-fold increase in IKβ promoter binding with TNFα.
- Background levels are significantly reduced in p65-HaloTag™ experiments.
- 25-fold fewer cells are needed using p65-HT than ChIP p65.

Conclusion

Overall, HaloTag™ technology provides increased efficiency, specificity, and sensitivity for the isolation of crosslinked protein:DNA complexes:

- Covalent capture results in decreased levels of background and higher signal-to-noise ratio as compared to the standard ChIP method.
- Binding of crosslinked HT:protein:DNA complexes to HaloLink is more rapid and specific than immunoprecipitation.
- Cell imaging, protein characterization, immobilization, and analysis of protein activity can all be achieved using HaloTag™ Technology.