HaloCHIP™ System

Protein:DNA Interaction Analysis

- Antibody-free
- Faster results compared to standard assays
- Covalent capture of intracellular protein:DNA complexes
- Improved signal-to-noise ratio

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Fast results

Obtain data quickly

Due to the rapid, covalent and specific binding of the HaloTag® fusion protein to the HaloLink™ Resin, the entire process from crosslinking to DNA release can be accomplished in 24 hours, compared to 5 days for standard ChIP. Minimizing the number of steps involved also reduces the risk of introducing experimental errors.

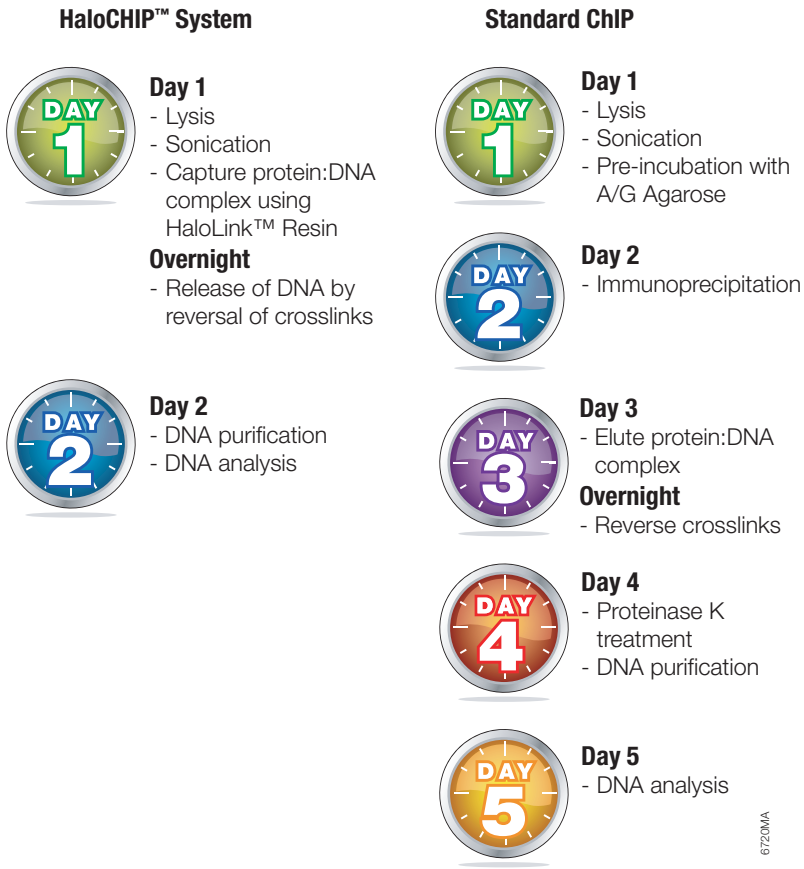

Figure 5. Comparison of time required for the HaloCHIP™ System and standard chromatin immunoprecipitation (ChIP) procedures.

Better than standard assays

Features

No requirement for antibody: No need to make your own or purchase expensive, qualified antibodies.

Obtain results faster: Obtain data in 24 hours with fewer steps to minimize potential experimental errors (Figure 5).

Improved signal-to-noise ratio: Enables detection of small changes in protein binding patterns using a minimal number of cells (Figure 3).

Complete protein characterization: HaloTag® Technology can also be used for imaging, protein immobilization and protein interaction analysis (Figure 2).

Table 1. Comparison of HaloCHIP™ System versus standard ChIP.

HaloCHIP™ System	Standard ChIP
No requirement for antibody	Antibody-based
1.5–2 days	4–5 days
Low levels of background, enhanced signal-to-noise ratio	High levels of background, low signal-to-noise ratio
Fewer steps, minimizing experimental errors	Multiple steps, resulting in variability of data
Covalent capture of protein:DNA complexes, enabling stringent washing and reducing background	Noncovalent capture of protein:DNA complexes
Tagged recombinant protein	Endogenous protein

Access to a broad range of applications

Complete protein characterization

Using a vector based on the HaloTag® Technology enables downstream applications such as cell imaging, immobilization and analysis of protein activity without having to reclone into specific vectors.

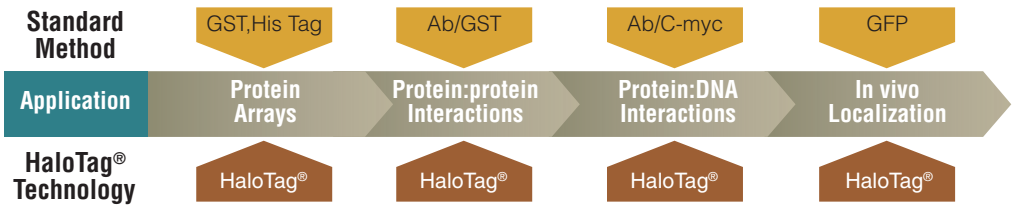

Figure 2. Global characterization of protein function and interactions using HaloTag® Technology.

Sensitive results without antibodies

Better signal, less background

Improved signal-to-noise ratio

The covalent binding of the crosslinked protein:DNA complexes to the resin enables stringent washing to remove non-specific DNA and proteins much more effectively than is possible by ChIP. Stringent washing effectively increases the signal-to-noise ratio to permit detection of small changes in protein binding within the genome.

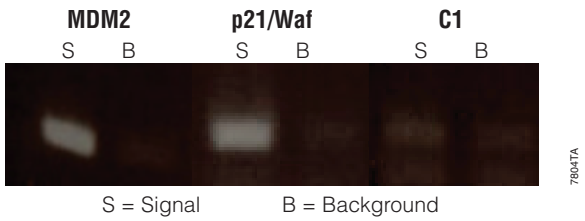

Figure 3. Sensitivity of the HaloCHIP™ System. Ethidium bromide-stained 2% agarose gels showing the PCR amplification of two p53 specific promoters, MDM2 and p21/Waf and one non-specific control promoter, C1, after using the HaloCHIP™ method. Untransfected or H1299 cells expressing p53-HaloTag were processed using HaloCHIP™ and the isolated DNA fragments purified and amplified using standard, endpoint PCR.

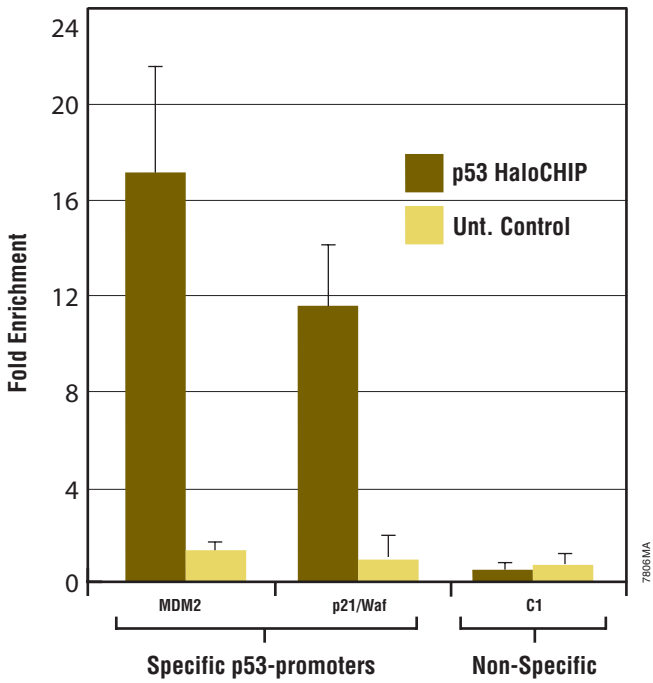

Figure 4. Enrichment of specific promoters in HaloCHIP. Plexor® quantitative PCR (qPCR) analysis of p53 HaloCHIP™ experimental and control samples, preparation described in Figure 3, showing the relative fold enrichment of p53-specific promoters, MDM2 and p21/Waf in comparison to a non-specific control promoter, C1. Using standard curves generated for each promoter, the absolute amount of all promoters within a single sample was calculated and fold-enrichment determined by dividing the amount of individual p53-specific promoters by the amount of C1 for each sample.

HaloCHIP™ System

Protein:DNA interaction analysis

The HaloCHIP™ System is a novel method designed for the covalent capture of intracellular protein:DNA complexes without the use of antibodies and offers an efficient and robust alternative to the standard chromatin immunoprecipitation (ChIP) method. Proteins of interest are expressed in cells as HaloTag® fusion proteins, crosslinked to DNA with formaldehyde, and then captured on HaloLink™ Resin, which forms a highly specific, covalent interaction with the HaloTag portion of the fusion protein. Due to the complete covalent linkage established between the resin and the crosslinked protein:DNA complexes, the resin can be stringently washed to remove nonspecific DNA and proteins much more effectively than is possible when using the ChIP method. The crosslinks are then reversed to release the purified DNA fragments from the HaloLink Resin (Figure 1).

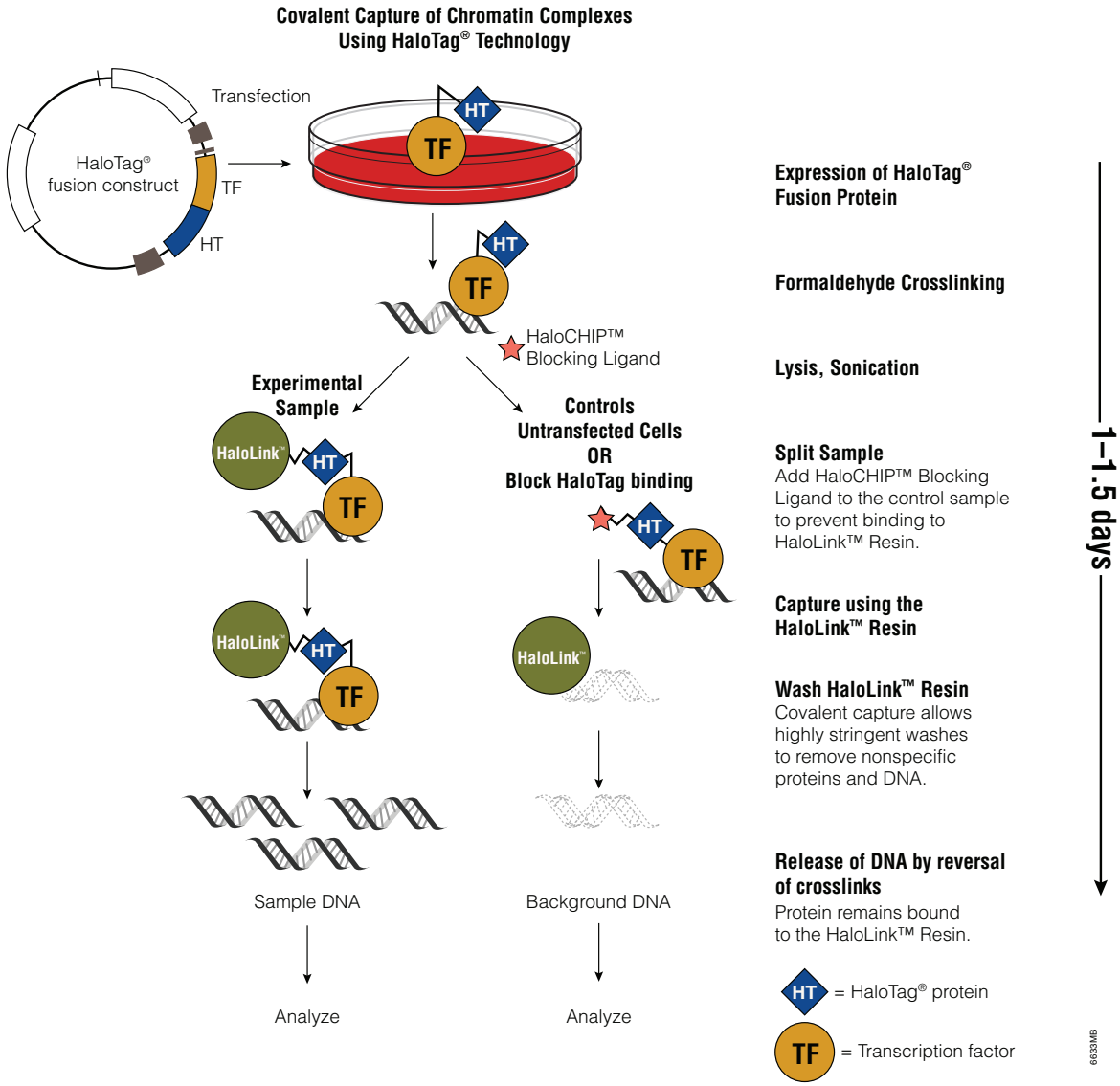

Figure 1. Schematic of HaloCHIP™ System procedure.

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Protein:DNA Interaction Analysis

Ordering Information

Product	Size	Cat.#
HaloCHIP™ System	20 reactions	G9410

Vector Name		
Product	**Size**	**Cat.#**
pFC14A (HaloTag® 7) CMV Flexi® Vector	20µg	G9651
pFC14K (HaloTag® 7) CMV Flexi® Vector	20µg	G9661
pFC15A (HaloTag® 7) CMV*d1* Flexi® Vector	20µg	G1611
pFC15K (HaloTag® 7) CMV*d1* Flexi® Vector	20µg	G1601
pFC16A (HaloTag® 7) CMV*d2* Flexi® Vector	20µg	G1591
pFC16K (HaloTag® 7) CMV*d2* Flexi® Vector	20µg	G1571
pFC17A (HaloTag® 7) CMV*d3* Flexi® Vector	20µg	G1551
pFC17K (HaloTag® 7) CMV*d3* Flexi® Vector	20µg	G1321
pFN21A (HaloTag® 7) CMV Flexi® Vector	20µg	G2821
pFN21K (HaloTag® 7) CMV Flexi® Vector	20µg	G2831
pFN22A (HaloTag® 7) CMV*d1* Flexi® Vector	20µg	G2841
pFN22K (HaloTag® 7) CMV*d1* Flexi® Vector	20µg	G2851
pFN23A (HaloTag® 7) CMV*d2* Flexi® Vector	20µg	G2861
pFN23K (HaloTag® 7) CMV*d2* Flexi® Vector	20µg	G2871
pFN24A (HaloTag® 7) CMV*d3* Flexi® Vector	20µg	G2881
pFN24K (HaloTag® 7) CMV*d3* Flexi® Vector	20µg	G2981
HaloTag® 7 Flexi® Vectors – CMV Deletion Series Sample Pack	9 x 2µg	G3780

For detailed product descriptions, please visit our Web site at: **www.promega.com**

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