Protein:Protein Interactions

with the HaloTag® platform

- Identify more physiologically-relevant protein partners
- Much lower background
- Transition from pull-downs into imaging studies with the same construct
- Simple customizable arrays for oriented protein immobilization
Mammalian pull-down protocol overview

1. Express HaloTag® fusion bait protein
2. Passive cell lysis
3. Rapid complex capture
4. Gentle washes
5a. TEV cleavage
5b. SDS Elution

1-2 Days
6-8 HOURS
**Study macro-molecular complexes**

Get consistent results and clean controls with HaloTag® when pulling down macro-molecular complexes.

The majority (26/29) of known subunits for the transcriptional coactivator complex, Mediator, were specifically isolated in a single pull-down experiment using MED26 as bait.

The HT control demonstrates the low non-specific binding of this fusion tag in pull-downs since 0/29 subunits of Mediator were identified.
Study simple (binary and tertiary) interactions

Expected cytoplasmic binary and tertiary protein interactions in the NFκB pathway were identified with specific p65-HaloTag® pull-down.

Proteins identified by MS
RelA(p65)
RelB
C-Rel
IκBα
IκBβ
IκBε
p100
p105(p50)
p52
Transition from pull-downs into imaging

No additional cloning is necessary to go between protein pull-down studies and cell imaging applications, as all HaloTag® Ligands are interchangeable.

U2OS cells stably expressing p65-HaloTag labeled with HaloTag TMRDirect™ Ligand show proper cytoplasmic localization.
**Identify new protein partners faster**

A single slide was used to study multiple protein:protein interactions.

Express HaloTag® fusion proteins in Tnt® cell-free lysates or mammalian cell lysates

Spot lysate (no pre-purification needed)

Wash

Immobilized HaloTag® fusion proteins

**Protocol**

1. **Expression of HaloTag® fusion proteins**
   - Express HaloTag® fusion proteins in Tnt® cell-free lysates or mammalian cell lysates.

2. **Spotting lysate**
   - Spot lysate (no pre-purification needed).

3. **Washing**
   - Wash immobilized HaloTag® fusion proteins.
**References**


**Ordering Information**

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<tr>
<th>PRODUCT</th>
<th>SIZE</th>
<th>CATALOG #</th>
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<tr>
<td>HaloTag® Mammalian Pull-Down and Labeling System</td>
<td>24 reactions</td>
<td>G6500, G6504</td>
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<tr>
<td>HaloLink™ Protein Arrays</td>
<td>various</td>
<td>G6140, G6180, G6190</td>
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