

S051 DUALhunter Plus Service Description

DUALhunter Plus screening for protein interactions involving self-activating proteins and nuclear or acidic proteins

What is the DUALhunter system?

The DUALhunter Plus system is a yeast-based, *in vivo* screening system to identify protein-protein interactions. This method is used to detect the protein interaction outside of the nucleus and therefore works with:

- All transcriptionally active proteins
- Any soluble protein
- Protein domains/ fragments

The DUALhunter Plus technology is based on the split-ubiquitin system originally invented by Nils Johnsson and Alexander Varshavsky (Johnsson and Varshavsky, 1994) and was developed by Dualsystems Biotech (Möckli *et al.*, 2007).

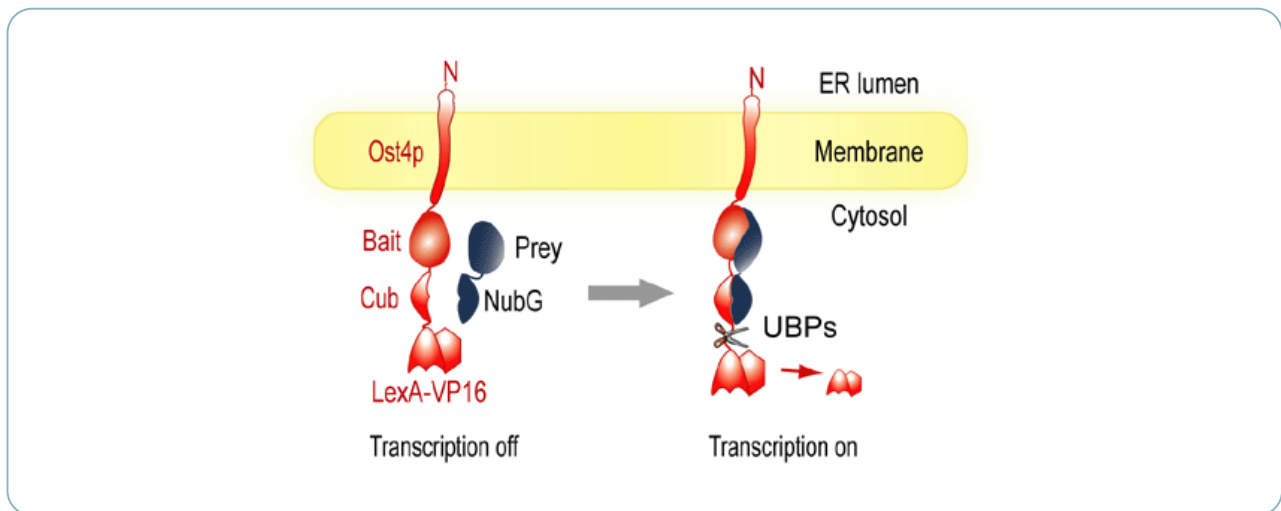
Why use a DUALhunter Plus screen?

By using our DUALhunter Plus screening services, you profit from our know-how in yeast-based screening:

- Go beyond classical yeast two-hybrid and screen self-activating proteins, transcription factors or acidic proteins
- No need to establish the DUALhunter Plus system in your lab
- Fast turnaround times
- You send us the cDNA encoding your protein of interest and select a cDNA library, we perform the entire screening for you
- Ready to use results, allowing you to immediately start downstream experiments

How does the DUALhunter system work?

The Figure below shows the mechanism of the DUALhunter Plus system. A protein of interest (the bait) is inserted between the membrane protein Ost4p and the C-terminal half of ubiquitin (Cub), followed by the artificial transcription factor LexA-VP16. A second protein (the prey) is fused to the mutated N-terminal half of ubiquitin (NubG).



The detection of a protein interaction is based on the split-ubiquitin system. If bait and prey interact, Cub and NubG complement to form split-ubiquitin, followed by cleavage and translocation of LexA-VP16 to the nucleus and transcriptional activation of endogenous reporter genes. The protein interaction between bait and prey is detected using the output of the reporter genes, either via a growth selection on minimal medium (-His/-Ade), or via the color marker lacZ. Replacing the plasmid encoding the prey by a cDNA library expressing millions of different proteins or protein fragments allows the uncovering of novel protein interactions.

How does a DUALhunter Plus screen work?

Part I: Bait construction and analysis

- We obtain the cDNA encoding your protein of interest from you and subclone your protein of interest into the bait vector of your choice
- A functional assay is performed to ensure that the bait is properly located within the membrane and is functional in the DUALhunter assay
- The bait is transformed into yeast and tested for toxicity. The autoactivation properties of the bait are also tested and the most appropriate selective pressure is determined.

Part II: Library screen and prey analysis

- The bait is screened by sequential transformation with a cDNA library of your choice
- Up to 384 prey clones are picked up and arrayed
- You receive a complete report including raw 5' and 3' prey sequences, prey identification by BLAST analysis and prey interaction domains.
- All results and any derived intellectual property fully belong to you

How much time does a DUALhunter Plus screen take?

- Part I 4-6 weeks
- Part II 8-10 weeks

DUALhunter literature

- Möckli *et al.* (2007) **BioTechniques** 42, 725-730

Ordering information

Order number	Custom service
P051	DUALhunter Plus custom screening service