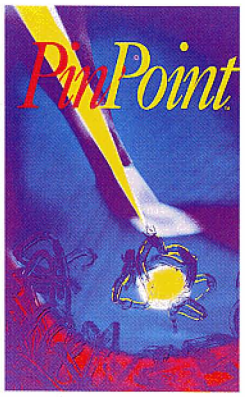


PinPoint™ The Pinnacle of Protein Expression and Purification

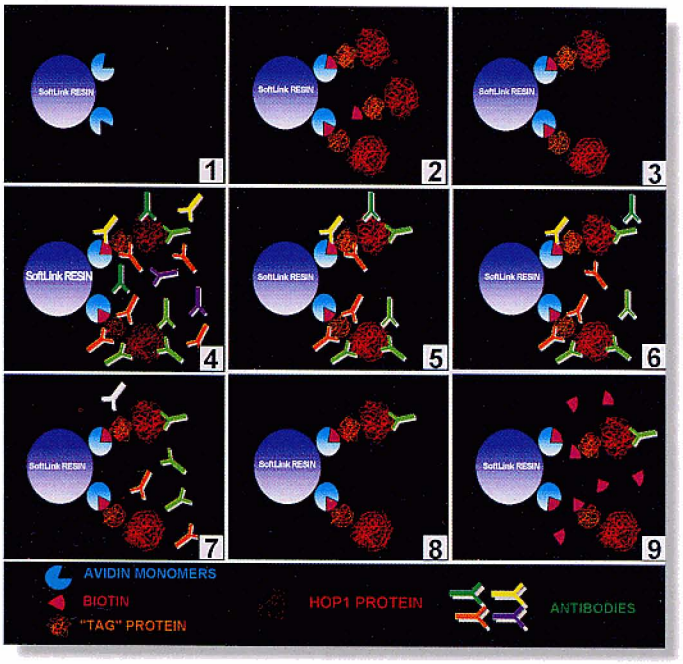
This is an expression and purification system for producing recombinant proteins in *E.coli* that are biotinylated during expression. This tag allows the detection and purification using the SoftLink™ Resin (soft release monomeric avidin matrix) under non-denaturing conditions. Large quantities of active protein can be purified using this system.

For more information about protein purification and expression systems please call Technical Direct on **0800–559900** or e-mail **uktechserve@uk.promega.com**

Using Promega's SoftLink™ Soft Release Avidin Resin to Affinity-Purify Antibodies to a Biotinylated *HOP1* Fusion Protein.

HOP1 gene of *Saccharomyces cerevisiae* is a meiosis-specific gene believed to play an important role in meiotic synapsis. In order to investigate the presence of plant proteins homologous to the *HOP1* product, the gene was subcloned into Promega's PinPoint™ Xa-2 vector, the overexpressed fusion protein purified by chromatography using SoftLink™ Soft Release Avidin Resin and finally polyclonal antibodies to the polypeptide were raised in rabbits.

In order to affinity-purify specific antibodies to the fusion protein, the IgG fraction of the antisera was processed as follows:



METHOD

1. Place 3ml of SoftLink™ resin in a plastic column and equilibrate using 30ml of 100mM potassium phosphate buffer, pH 7.0 (equilibration buffer), setting a low flow rate (0.5 ml/min).
2. Apply the biotinylated *HOP1* fusion protein dissolved in equilibration buffer. An excess of the protein (ca. 10mg) was used in order to ensure saturation of binding sites in the resin. Also for this purpose, the protein-containing solution was re-applied 3 times.
3. Wash the column sequentially with 30ml of the following:
   Equilibration buffer.
   100mM glycine, pH 2.5 (eluting solution #1).
   Equilibration buffer.
   100mM triethylamine, pH 11.5 (eluting solution #2).
   Equilibration buffer.
4. Apply the IgG fraction corresponding to 1.5ml of crude *HOP1* antiserum and recirculate the sample three more times.
5. Wash the column with 30ml of equilibration buffer.
6. Elution of antibodies I: Apply 30ml of eluting solution #1 and retain the flow-through solution. Equilibrate using 30ml of equilibration buffer.
7. Elution of antibodies II: Apply 30ml of eluting solution #2 and retain the flow-through solution. Equilibrate the resin with 30ml of equilibration buffer for re-use.

8/9. The fusion protein is retained by the column under the conditions used and can be eluted itself by incubating the resin with 10mM Tris-HCl buffer (pH 7.5) containing 5mM biotin for 15 minutes.

AUTHOR'S NOTES

- Eluted antibodies can be pooled and further concentrated using either ammonium sulphate or ultrafiltration. As a final step of this concentration, change buffer to PBS.
- Antibody yield is dependant on the initial titre and specificity of the antibodies in the crude serum. Also the use of alternative eluting methods (i.e. concentrated solutions of $MgCl_2$, potassium thiocyanate, or NaSCN) capable of eluting the bound antibodies without eluting the fusion protein could be applied to produce a higher yield.
- The column maybe reused for at least three more cycles of purification without appreciable loss in binding capacity. However, lower yields would be expected from extended usage, as non-eluted antibodies may remain attached to the column, blocking the recognition sites for the antibodies (Fig 8).
- The resulting antibodies have been tested in a number of applications including immunoblotting and immunocytochemistry. They display a high specificity and are able to recognise *HOP1* fusion protein, the natural protein expressed in yeast and also cross-reactive proteins in plants. However, background is significantly reduced when compared to both the crude serum and its IgG fraction.

We would like to thank Dr Juan de Dois Alché for supplying these results based on his use of SoftLink™.

Dr. Juan de Dois Alché and Prof. Hugh Dickinson
Plant Sciences Department.
University of Oxford
South Parks Road
Oxford, OX1 3RB

REFERENCES

Hollingsworth, N. M., Goetsch, L., Byers, B. (1990). The *HOP1* gene encodes a meiosis specific component of yeast chromosomes. Cell 61:73-84.
Alché, J. D., Dickinson, H. G. (1998). Affinity chromatographic purification of antibodies to a biotinylated fusion protein expressed in *Escherichia coli*. Prot. Expres.Purif. 12: 138-143.